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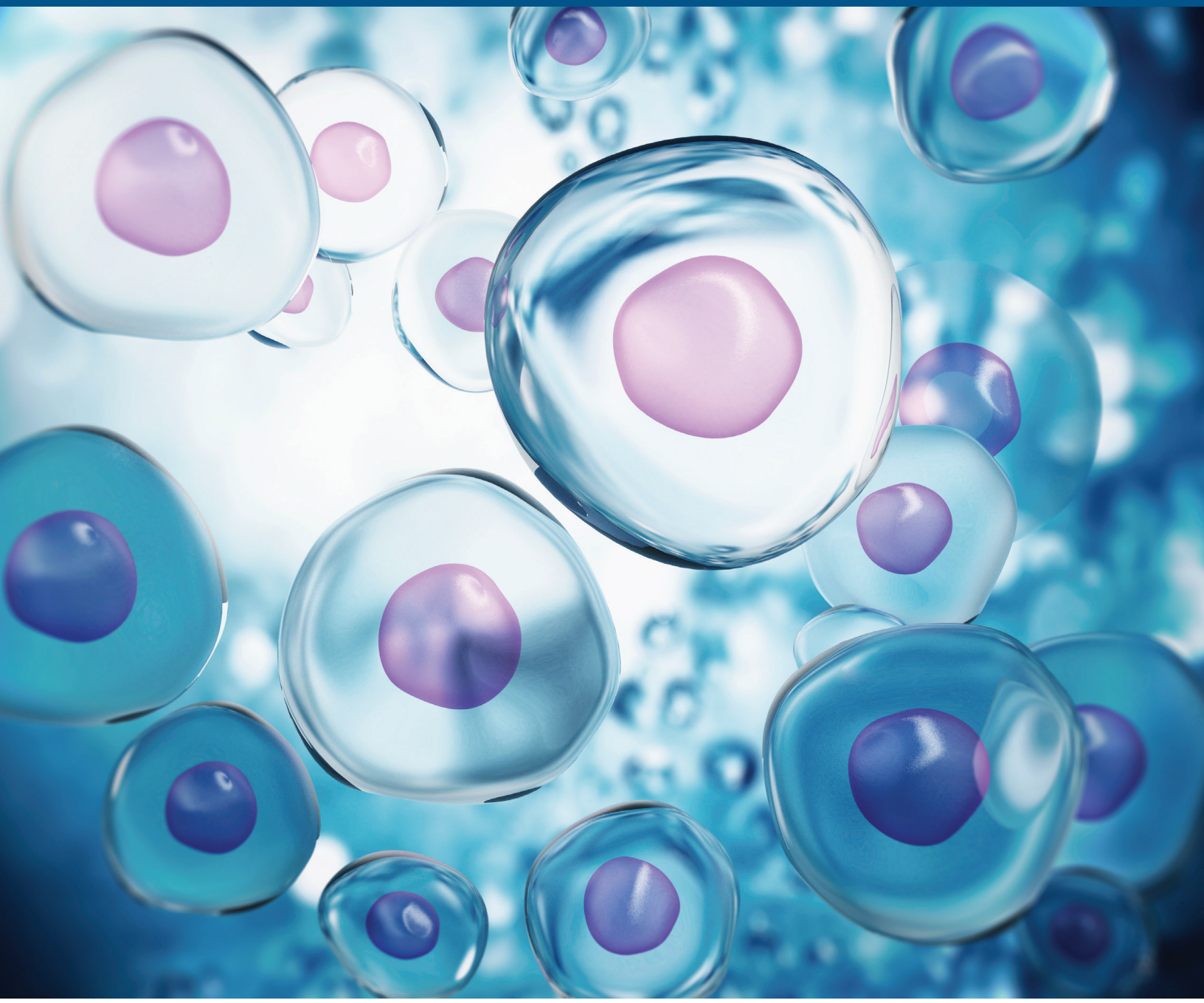


Gibco BioProduction Services

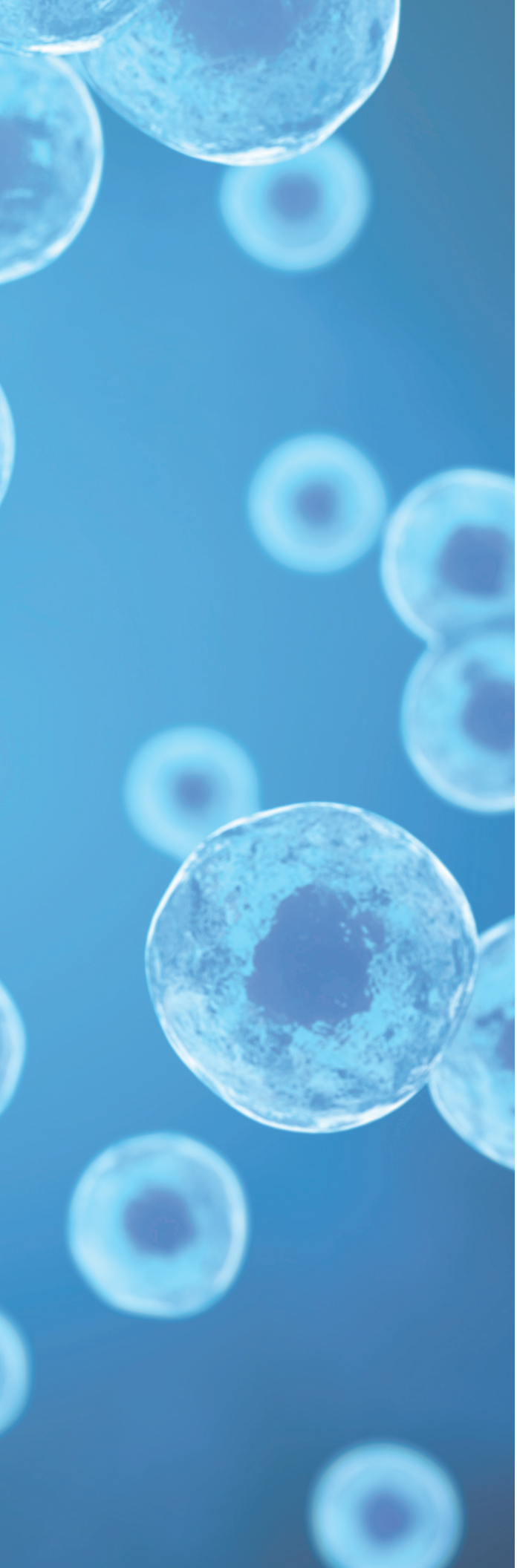
Analytical services to help you get the most from your cell culture

ThermoFisher SCIENTIFIC

Shorten your time-to-market by letting our experienced team of analytical scientists help you understand how to get the most from your cell culture. Gibco™ BioProduction Services offers a full suite of customized analytical services as part of our customization continuum. These services offer powerful and insightful data that can help you save time and money throughout your workflow.

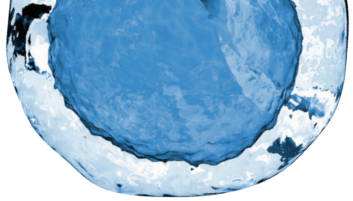
Our analytical services include spent media analysis and protein quality analysis. By analyzing a small sample of your medium, we can help you optimize for increased titers, productivity, and quality as well as troubleshoot issues. If you need additional support for media stability, we can also help you to understand the integrity of your Gibco™ medium at certain points in time and under certain conditions. Upon completion of these services, you will receive an easy-to-read quantitative report of the results. If desired, a consultation is available for protein analysis and spent media analysis at no extra charge.

We have been helping clients accelerate their development and process time using our analytical services for more than 15 years. Our offerings are customized to your unique needs and simple to order. Save time and hassle by partnering with an experienced team that you can trust with your samples.



Contents

Spent media analysis



Are you looking to perform media screening and development, bioreactor runs, process optimization, clone screening and selection, or stability experiments? If so, Gibco BioProduction Services can help you accelerate your time-to-market by assisting you in understanding the nutrient needs of your unique cell culture system. This is crucial to optimizing your media. We offer rapid testing along with consultation to help optimize your media.

Our team of experienced scientists can analyze levels of amino acids, water-soluble vitamins, metabolites, and trace elements in samples taken at different time points of your process. You have the flexibility to customize your request to include the specific analyses you desire, as we believe in offering you the freedom to choose exactly what you need. Many of our tests require a sample size of only 1 mL of spent media.

We have worked with most platforms, including CHO, yeast, insect, and stem cells, and are ready to support you in characterizing your cultures.

Benefits of spent media analysis

- Turnaround time of 10 business days for analysis of amino acids and water-soluble vitamins
- Access to experienced scientists for consultation
- Samples tested in multiple facilities located in the United States, Scotland, and China; depending on your unique needs, samples can be submitted to any of the three locations (Table 1)
- Flexible terms and conditions

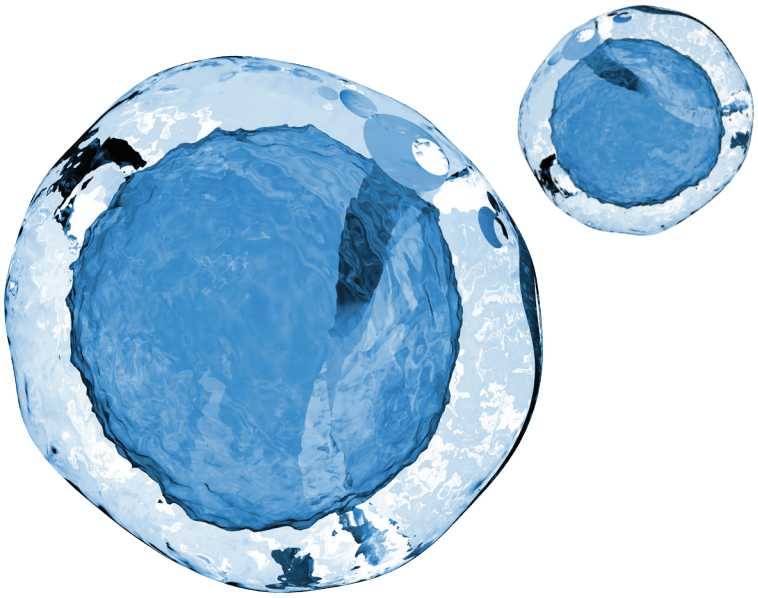


Table 1. Spent media analytical services.

	Amino acids	Water-soluble vitamins	Advanced water-soluble vitamins	Glucose, lactate	Insulin	Pluronic surfactant	Cholesterol	GlutaMAX Supplement	IgG	Glycans	Charge variants	Trace elements	Orthophosphate
Grand Island	+	+		+	+	+	+	+	+	+	+	+	+
Paisley	+		+	+	+	+	+	+				+	+
Shanghai	+	+		+				+					

What can we test for?

Our analytical services team can test your spent media sample for a number of amino acids, water-soluble vitamin components, and trace elements (Table 2).

Table 2. Tested components.

Amino acids		Water-soluble vitamins	Advanced water-soluble vitamins
• Ammonia	• L-isoleucine	• Cyanocobalamin (B-12)	• Biotin
• L-alanine	• L-leucine	• Folic acid	• Cyanocobalamin (B-12)
• L-arginine	• L-lysine HCl	• Niacinamide	• Folic acid
• L-asparagine	• L-methionine	• Riboflavin	• Niacinamide
• L-aspartic acid	• L-phenylalanine	• Thiamine HCl	• Pantothenate
• L-cystine, L-cysteine	• L-proline	Additional components:	• Pyridoxal
• Ethanolamine HCl	• L-serine	• Phenol red	• Pyridoxine
• L-glutamic acid	• L-threonine	• L-tryptophan	• Riboflavin
• L-glutamine	• L-tryptophan		• Thiamine HCl
• Glycine	• L-tyrosine		Additional components:
• L-histidine	• L-valine		• Phenol red
• L-hydroxyproline			• L-tryptophan

Trace elements				
• Antimony	• Cobalt	• Lithium	• Ruthenium	• Tin
• Arsenic	• Copper	• Magnesium	• Selenium	• Titanium
• Barium	• Gallium	• Manganese	• Silicon	• Tungsten
• Beryllium	• Germanium	• Molybdenum	• Silver	• Uranium
• Boron	• Gold	• Nickel	• Sodium	• Vanadium
• Cadmium	• Hafnium	• Palladium	• Strontium	• Zinc
• Calcium	• Iron	• Potassium	• Tantalum	
• Chromium	• Lead	• Rhenium	• Thallium	

In addition, we offer analysis for glucose and lactate, insulin, Pluronic™ surfactant, cholesterol, Gibco™ GlutaMAX™ Supplement, IgG, and orthophosphate. We use a variety of testing techniques that enable highly sensitive results (Tables 3 and 4).

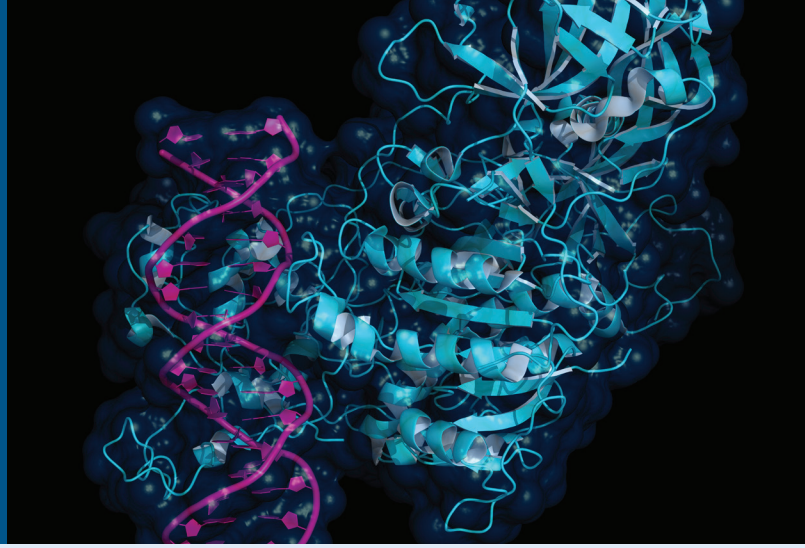
Table 3. Testing techniques.

Analysis	Technique
• Amino acids	• Ultrahigh-performance liquid chromatography (UHPLC)
• Water-soluble vitamins	• High-performance liquid chromatography (HPLC)
• Advanced water-soluble vitamins	• UHPLC
• Glucose, lactate	• Bioanalyzer™ system
• Insulin	• HPLC, UHPLC
• Cholesterol	• Gas chromatography
• GlutaMAX Supplement	• UHPLC
• IgG	• HPLC
• Glycans	• Capillary electrophoresis
• Charge variants	• HPLC, UHPLC
• Trace elements	• Inductively coupled plasma-mass spectrometry (ICP-MS)
• Orthophosphate	• Ion chromatography, colorimetric assay
• Pluronic surfactant for fresh media or stability studies	• HPLC, UHPLC

Table 4. Limits of detection.

	Sample volume			
Analysis	Recommended	Minimum	**Limit of detection**	**Standard lead time**
Amino acids	500 µL	200 µL	Varies by component, but all are ~10 µM	10 business days
Water-soluble vitamins	2 mL	0.7 mL	Varies by component, but all are <1 µM	10 business days
Glucose, lactate	500 µL	200 µL	<1 µM	10 business days
Insulin	2 mL	0.5 mL	<0.1 µM	10 business days
GlutaMAX Supplement	500 µL	200 µL	<5 µM	10 business days
Pluronic surfactant (fresh medium only)	500 µL	200 µL	60 mg/L	~15 business days
Orthophospate	10 mL	2 mL	1 mg/L	~15 business days
Cholesterol	5 mL	3 mL	<1 µM	~15 business days
Trace elements	50 mL	5 mL	Varies by element and sample volume	~15 business days

Case study: spent media analysis
The following case study shows how spent media analysis results were used to identify which supplier's medium resulted in the best lactate accumulation profile.



Situation

- Many base media contain components that result in lactate accumulation.
- Gibco BioProduction Services sought to confirm that use of our Gibco™ Dynamis™ Medium would yield the best lactate profile in the cell culture.

Our response

- Our scientists used metabolite analysis of spent media to confirm the ability to reduce lactate accumulation.
- Analysis was performed on our Dynamis Medium and several media from other suppliers (Figure 1).

Value delivered

- Identification of lowest lactate accumulation with Dynamis Medium, among all media tested.

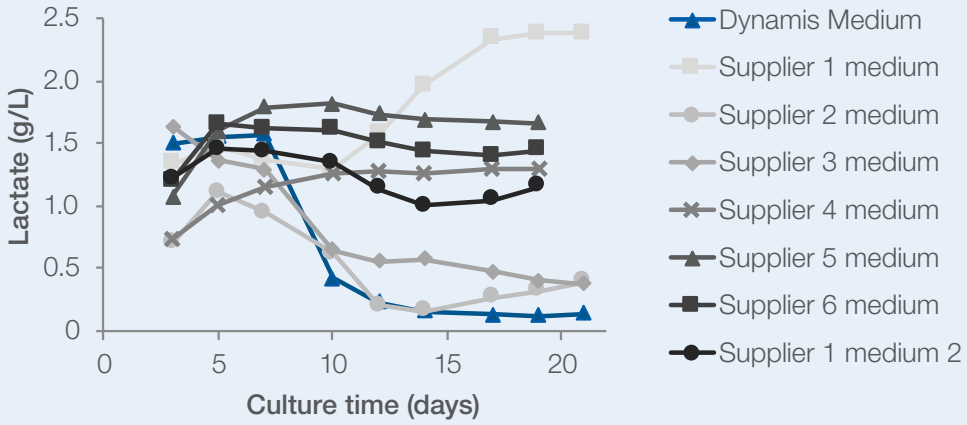


Figure 1. Low accumulation of lactate in DG44 culture with Dynamis Medium.

Easy-to-read results

Upon completion of your project, you will be provided quantitative results in an easy-to-read spreadsheet. The format allows for easy manipulation should you desire to customize the output style, and we can provide consultation at no additional cost to discuss the best path forward to optimization. Figure 2 shows an example of how your results will look.

REQUESTED BY:				
PROJECT:				
DATE OF REQUEST:				

Sample Details				
DESCRIPTION	>			
SKU or DAY/PASSGE	>			
LOT NO.	>			
SAMPLE NUMBER	>			
Component			**mg/L**	
L-ALANINE				
L-ARGININE				
L-ASPARAGINE H2O				
L-ASPARTIC ACID				
L-CYSTINE *				
ETHANOLAMINE HCl				
L-GLUTAMINE				
L-GLUTAMIC ACID				
GLYCINE				
L-HISTIDINE				
HYDROXY-L-PROLINE				
L-ISOLEUCINE				
L-LEUCINE				
L-LYSINE HCl				
L-METHIONINE				
AMMONIA				
L-PHENYLALANINE				
L-PROLINE				
L-SERINE				
L-THREONINE				
L-TRYPTOPHAN				
L-TYROSINE				
L-VALINE				

B-12			
FOLIC ACID			
NIACINAMIDE			
PHENOL RED			
RIBOFLAVIN			
THIAMINE HCl			
L-TRYPTOPHAN			

Figure 2.

How to get started

We keep our process simple and easy (Figure 3). In fact, we only require three actions of you to get started:

- Request a quote
- Generate a purchase order (PO)
- Return the signed PO with submission form and samples

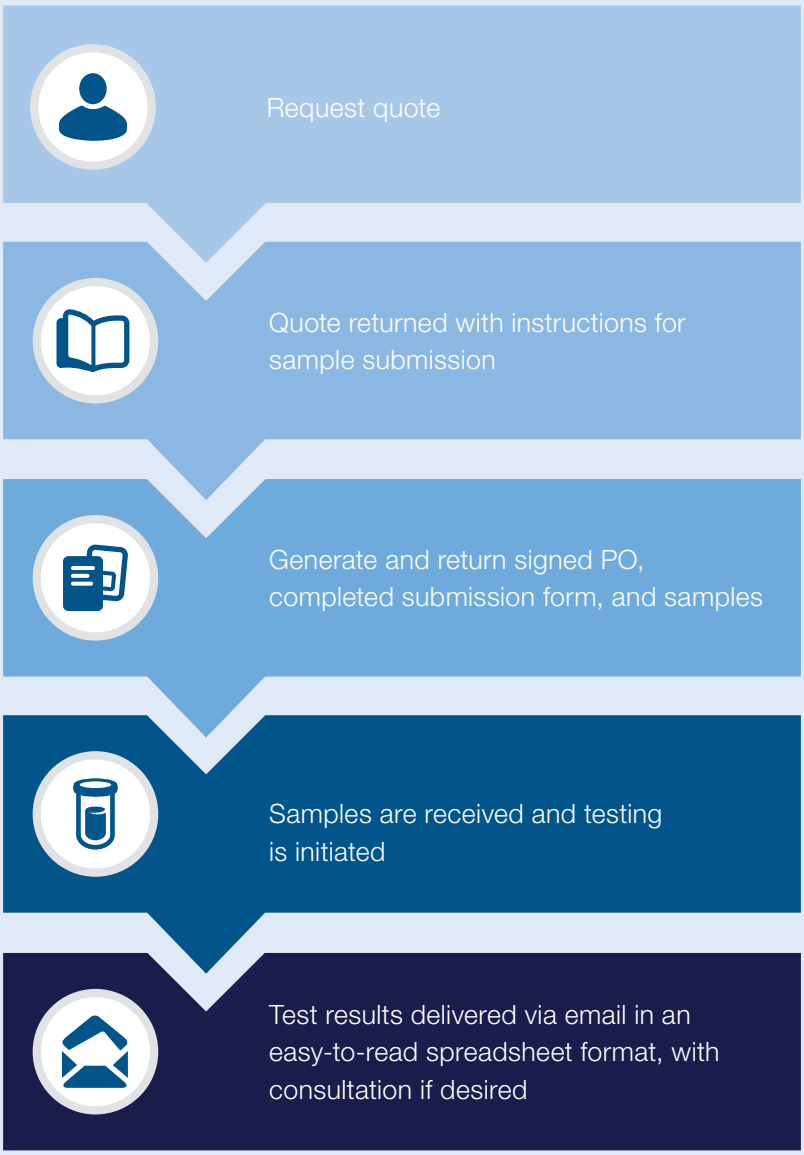


Figure 3. Overall process for analytical service.

Protein quality analysis

Gibco BioProduction Services offers the following analytical services to help you assess protein quality:

- Glycan analysis
- Charge variant analysis

Glycan analysis

The glycosylation profile of a recombinant protein product is one of the most important attributes when defining product quality. Glycosylation changes can have a profound impact on the safety and efficacy of a biological drug. We are ready to assist you with identifying changes in your protein's N-glycan patterns or aberrant N-glycan patterns in antibodies.

We use instruments and reagents from Thermo Fisher Scientific that were developed specifically for N-glycan analysis. Our instruments are highly sensitive and can analyze complex glycan mixtures with a sample size as small as 50 µg of purified protein sample. We can help you begin the path to optimization and acceleration to market. Upon completion of your N-glycan analysis, your local field applications scientist (FAS) can meet with you to discuss the results and how we can further assist you with modulating the glycosylation profile to match your protein of interest.

Benefits of glycan analysis

- Expedited process development
- Improved pharmacokinetics of molecule

How do we test?

We use a high-throughput screening technique that allows us to test over 100 samples at once in an analysis run. Using this method enables us to maximize throughput and shorten the time needed to prepare, analyze, and report on a large number of samples. We are able to achieve this testing in as little as 48 hours.

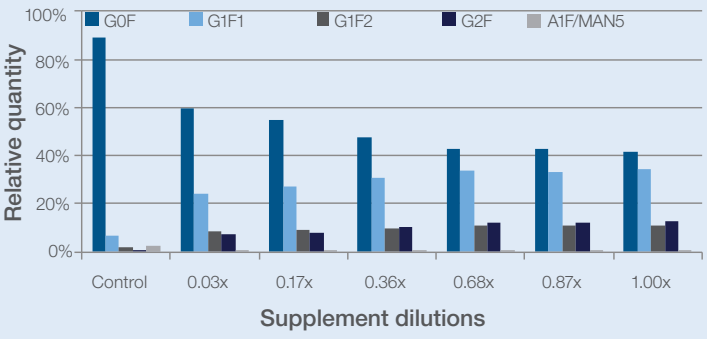
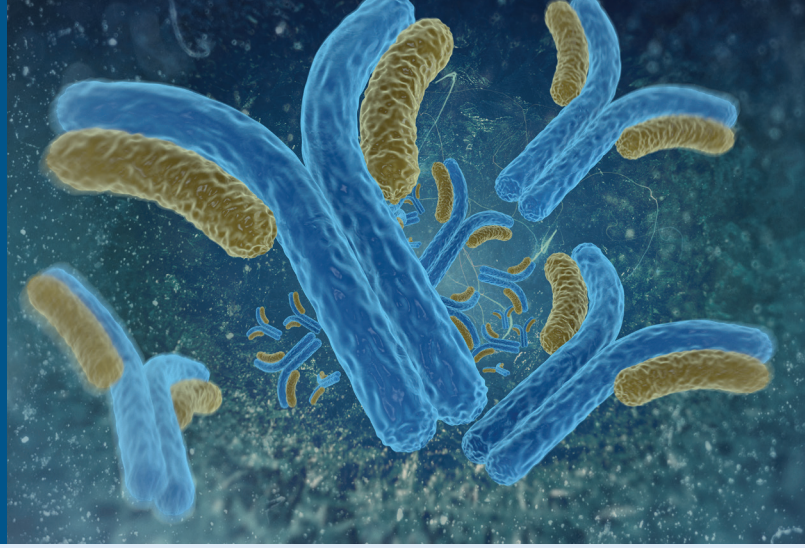


Figure 4. Using a supplement-based approach to modulate glycosylation profiles results in only partial modulation. Glycan analysis results for DG44 are shown as the percentage of total glycans. Using a glycosylation-enhancing supplement (dilutions derived from a design-of-experiments (DOE) approach) added on days 3, 5, and 7 results in a shift from G0F to G1F and G2F. While there is an increase in terminal galactosylation, fine-tuning the concentration of glycosylation-enhancing components in the supplement to target specific glycan profiles throughout the entire G0F range (90% to 40%) left a 30% gap in G0F. Using the supplement-based approach, it was not possible to target glycan profiles between 90% and 60% G0F between the control and 0.03x conditions.

Case study: modulation of glycan profiles
The following case study shows how glycan analysis was used to modulate glycan profiles while maximizing cell growth and antibody titer.



Situation

- Traditionally, the approach used to modify the glycosylation profiles of proteins involved supplementing a culture with components that could improve galactosylation; experimentation using this approach resulted in a dramatic increase in terminal galactosylation, but lacked the ability to target specific glycosylation profiles through the entire spectrum.
- Using a DG44 cell line, we wanted to demonstrate that we could maximize growth and titer while being able to modulate glycan profiles using our proprietary feed and unique feeding process.

Our response

- Capillary electrophoresis–based glycan analysis on the Applied Biosystems™ 3500 Genetic Analyzer was used to determine glycan profiles using the traditional, supplement-based approach (Figure 4).
- We then used our proprietary method with Gibco™ EfficientFeed™ C+ and GlycanTune™ C+ feeds to target the glycan profile (Figure 5).

Value delivered

- We were able to maximize growth and titer while modulating glycan profiles, using EfficientFeed C+ and GlycanTune C+ feeds in conjunction with our unique feeding process.

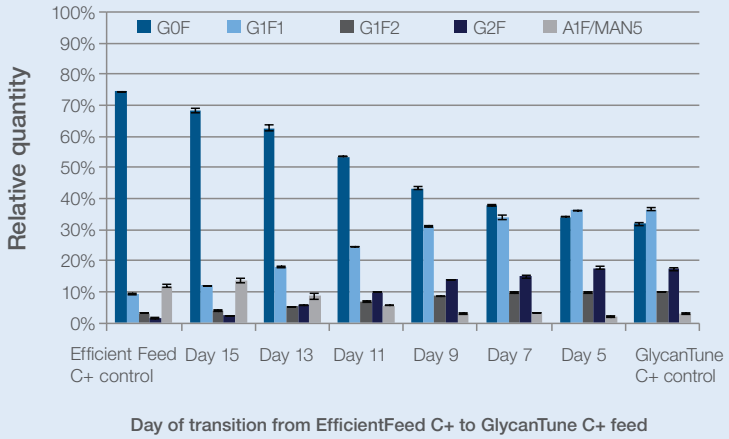
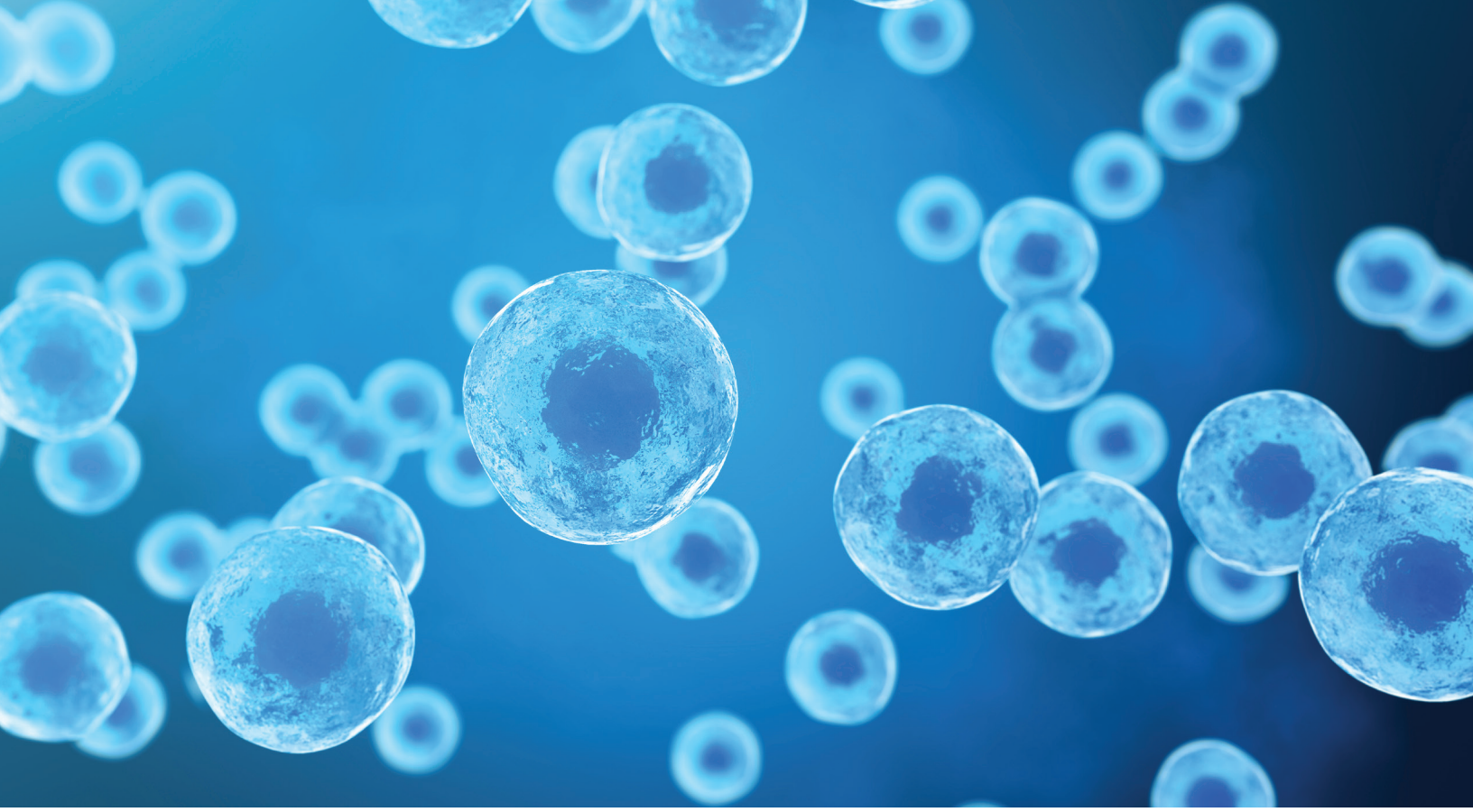


Figure 5. Glycan analysis from modulating glycosylation with EfficientFeed C+ and GlycanTune C+ feeds. The timing of transition from EfficientFeed C+ to GlycanTune C+ feed makes it possible to target specific glycosylation profiles. G0F was modulated from 75% down to 32% while increasing G1F (1 and 2) and G2F.



Charge variant analysis

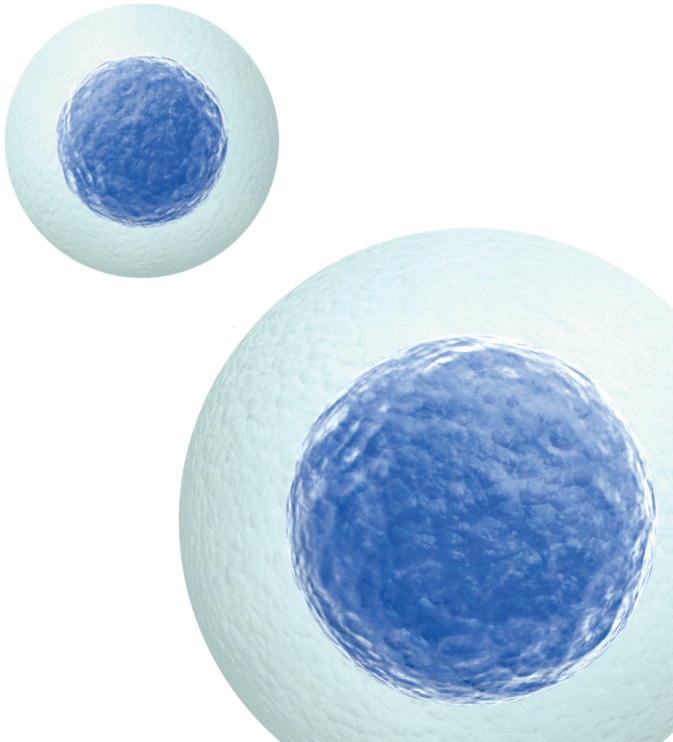
Understanding the profile of your molecule is crucial to identifying charge variants and modifying them if necessary. Charge heterogeneity can change throughout the biopharmaceutical production process as well as during purification and storage. These changes can impact the yield, structure, stability, and biological function of the protein. Because of this, it is important to characterize and monitor the charge variant profile of your molecule, not only over the course of the biopharmaceutical production process but also at lot release. Our team of experienced analytical scientists can assist you in understanding the charge variability of your antibody at different time points of your process. Maintaining consistency of balanced positive and negative charges is essential for optimal performance of your molecule.

Benefits of charge variant analysis

- Helps increase downstream yields
- Provides important information regarding product quality
- Helps simplify downstream processes
- Helps save time and resources

How do we test?

We use Thermo Scientific™ MAbPac™ SCX-10 columns and CX-1 pH gradient buffers to test proteins for charge variants. We employ UHPLC, which enables us to use small sample sizes. With a working range of 5–100 µg of purified product per sample, we can test your protein for charge variants.



Stability studies

Understanding the stability of your Gibco™ custom medium formulation over time is important for setting expiration dates for materials in your bioproduction process. We can assist you in generating such data. We offer real-time stability testing of your Gibco™ catalog and custom products in their packaged form, under certain conditions and at specific time points determined by you.

As the manufacturer of your media, we have specialized knowledge of your custom formulation. By outsourcing your stability testing to us, you eliminate the time and steps required to transfer lots. In addition, you eliminate the need for space to store the equipment and personnel to run the tests. Our team of experienced scientists can customize a stability protocol to match your requirements to meet your internal quality needs. We have performed testing for industry, government, and academia. For your convenience, testing is performed at both our North American site in Grand Island, New York, and our European site in Paisley, Scotland.

Benefits of outsourcing your Gibco medium stability studies to Thermo Fisher Scientific

- No need to transfer samples from multiple lots
- Specialized knowledge to apply to your formulation

What types of conditions can we test?

We can assess the shelf life of your formulation under a number of conditions (Table 5).

Table 5. Conditions for stability tests.

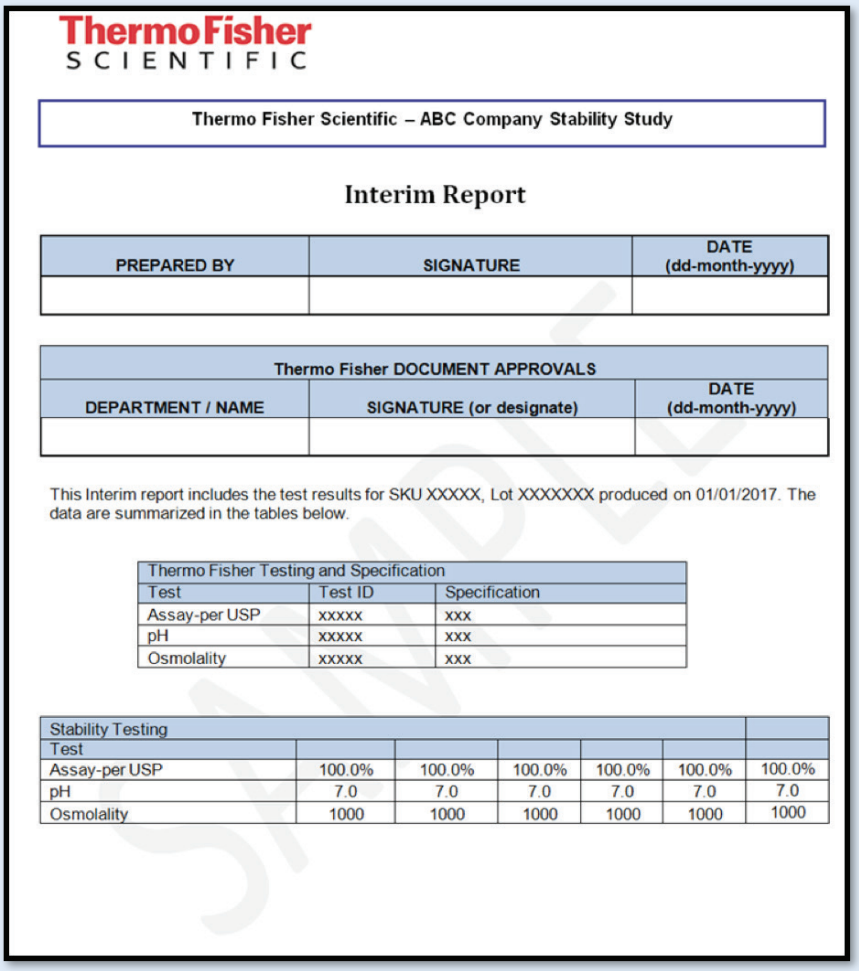
Test area	Condition
Format	Advanced Granulation Technology (AGT™), dry powder, or liquid medium
Intended use	Research use only (RUO), *in vitro* diagnostic (IVD), other
Storage temperature	–20 to –5°C, 2 to 8°C, 15 to 30°C
Protection from light	Yes or no
Excursion temperature, if applicable	Short-term conditions at higher or lower temperature: –20 to –5°C, 2 to 8°C, 15 to 30°C
Excursion condition, if applicable	Light or dark

Quality attributes

We have the capability to perform a variety of quality tests depending on the stability factors indicated for your unique formulation. While pH and osmolality are stability-indicating factors for most products, endotoxin, mycoplasma, bioburden, and other tests may be included based on your unique formulation. However, the list of assays is ultimately dictated by you. We also have the capability to perform analytical testing as part of our stability program, if applicable. For a more exhaustive list of assays, please speak with your bioproduction account manager.

Results

Our reports are easy to follow and interpret. Figure 6 shows a sample of our easy-to-read report that is provided at an interim time point. The final report follows the same format.



ThermoFisher SCIENTIFIC

Thermo Fisher Scientific – ABC Company Stability Study

Interim Report

PREPARED BY	SIGNATURE	DATE (dd-month-yyyy)

Thermo Fisher DOCUMENT APPROVALS		
DEPARTMENT / NAME	SIGNATURE (or designate)	DATE (dd-month-yyyy)

This Interim report includes the test results for SKU XXXXX, Lot XXXXXXX produced on 01/01/2017. The data are summarized in the tables below.

Thermo Fisher Testing and Specification		
Test	Test ID	Specification
Assay-per USP	xxxxx	xxx
pH	xxxxx	xxx
Osmolality	xxxxx	xxx

Stability Testing						
Test						
Assay-per USP	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
pH	7.0	7.0	7.0	7.0	7.0	7.0
Osmolality	1000	1000	1000	1000	1000	1000

Figure 6. Sample results from stability service.

How to get started

Getting started is easy. You only need to set the test specifications. From here, our team will determine feasibility and draft the protocols for your review and acceptance (Figure 7).

Figure 7. Overall process for stability testing service.

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Whatever your analytical need, the Gibco BioProduction Services team is here to assist you. Not only will we help you get the most from your cell culture, but we will also assign a certified project manager who will act as your single point of contact for your analytical project. It's not just what we do that's important, but how we do it as well, which is why we've designed our processes to be customized, convenient, transparent, and rapid. We understand the importance of having an optimized end solution that will enable you to get to the marketplace sooner. We are ready to partner with you—let us help you accelerate your path from discovery to market.

Grand Island, New York

Shanghai, China

Paisley, Scotland

	Glycan analysis	Charge variant analysis	Spent media analysis	Stability studies
Grand Island (US)	+	+	+	+
Paisley (Europe)			+	+
Shanghai (China)			+	

For more information regarding any of our analytical offerings, or to begin a project today, please contact your bioproduction account manager or email us at **gibcoservices@thermofisher.com**

For more information about Gibco BioProduction Services, please visit **thermofisher.com/gibcobpdservices**

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