


## Technologies for Monoclonal Antibody Production





**First we help you bridge the distance  
between upstream and downstream.**

**Then we help you shorten it.**

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Monoclonal antibodies for therapeutic, diagnostic and industrial applications represent as many as one-third of all biotechnology products currently in development.

As a manufacturer of biomolecules, you face an ever-burgeoning demand for biotechnology-based proteins. You are always searching for better answers to the myriad challenges of process development—practical, proven solutions that will help you bring products to market more quickly and efficiently.

Upstream to downstream, Invitrogen can provide you with those solutions: GIBCO™ products for cell culture and our comprehensive expertise in process integration and optimization.

Interested in learning how our capabilities can help enhance yours? Call us to arrange a seminar at your site. Together we will share experiences and objectives, exploring total system integration to focus on a common goal: increasingly efficient antibody production.

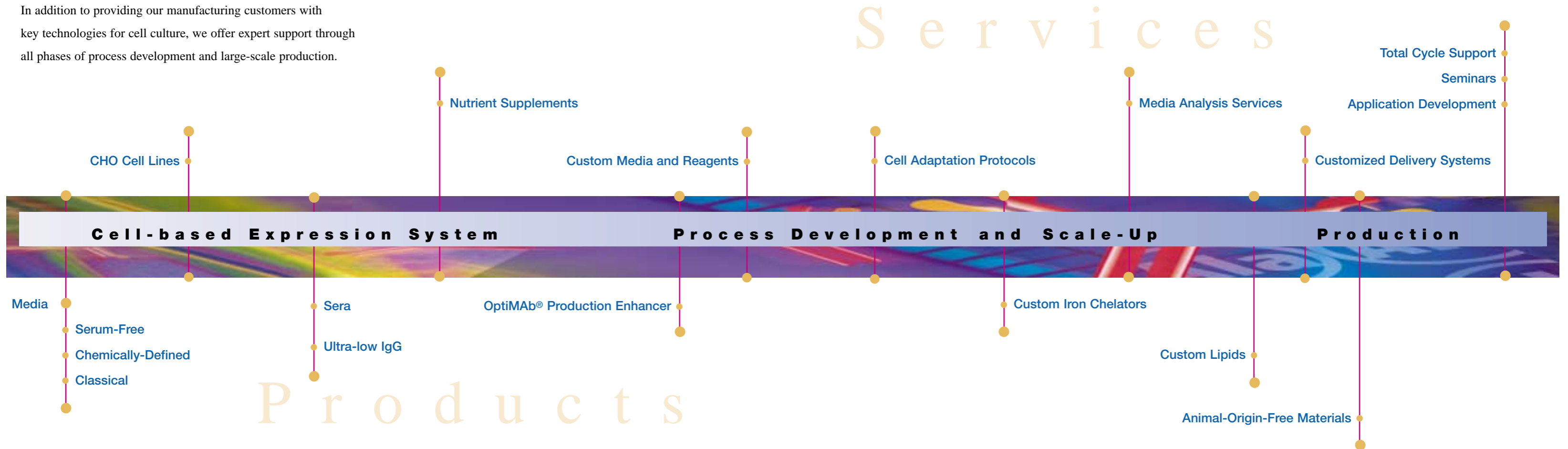


## You can rely on us to integrate and optimize your processes.

Invitrogen is the world's foremost supplier of products, services and technologies for life science research and biomanufacturing.

Leaders in the biotechnology, pharmaceutical, agricultural and chemical industries rely on us for help in getting their products to market faster.

In addition to providing our manufacturing customers with key technologies for cell culture, we offer expert support through all phases of process development and large-scale production.



### Minimizing Risk

As you move from research to process development and into large-scale manufacturing, your concerns about the presence of animal-origin materials in cell culture products grow exponentially.

We can help you develop processes that pose as few regulatory concerns as possible, balancing your requirements for performance with your requirements for safety and compliance.

Worldwide, we are implementing the industry's most comprehensive strategy for minimizing or eliminating animal-origin materials.



### In-Depth Support

In addition to providing you with the industry's finest training and technical support, we are also able to offer you access to our expertise in R&D, manufacturing, process optimization, and regulatory affairs.

As you enjoy an in-depth consultative relationship with Invitrogen, you can call upon many of our specialized internal resources including our best scientists and engineers.



### Custom Manufacturing and Services

With dedicated customer service, technology transfer and manufacturing personnel, our Custom Manufacturing Services Group facilitates the design, sourcing and production of products and technologies for your specific needs.

We can provide you with custom services that include manufacturing of custom media, sera and related products; media optimization; custom manufacturing, process design and specification development; standard and specialized product testing; and multiple options in lot sizes and packaging.



### Global Operations

Wherever you may be, you can always depend on Invitrogen to supply what you need when you need it. Our global sourcing and manufacturing capabilities, combined with local service, assure you of timely and uninterrupted delivery of products, services and support.

## Key Technologies for MAb Production

Upstream decisions usually affect downstream production—  
for better or worse.

When you choose Invitrogen's GIBCO™-brand products, you will have access to our resources to support your process development. Then, as you scale up, we will work to meet your needs for large-scale monoclonal antibody production and purification by modifying or customizing our technologies.

### Innovations in Cell Culture

The largest manufacturer of cell culture media in the world, Invitrogen has developed new nutrient media and other innovative products appropriate for the production of biomolecules. These products are optimized to minimize adaptation time from existing cultures and to simplify the scale-up process.

Your choice of our newest generation of GIBCO™ media, combined with the use of new supplements, will provide greater control and growth rates, and higher yields than any other method. It will also facilitate downstream purification.

### CHO Media and Cell Lines

Chinese Hamster Ovary cells (CHO) are commonly used for expression of recombinant proteins, including monoclonal antibodies. The ability to grow to high density in serum-free suspension culture conditions, as well as to express and secrete proteins with the appropriate post-translational modifications (e.g., glycosylation), make CHO cells the choice for production of many antibodies or proteins intended for human therapeutic applications. (See figure 1.)

Recombinant CHO cells can successfully grow in large-scale cultures of either adherent cells or suspension-adapted cells. The trend toward use of serum-free, protein-free and chemically-defined culture conditions has made it more difficult to use attachment dependent CHO cells and has resulted in an increased use of suspension-adapted cells.

We have developed several GIBCO™ serum-free media formulations for use in large-scale suspension culture applications:

- CD CHO Medium is a protein-free, chemically-defined medium without L-glutamine, hypoxanthine or thymidine. It is made without any components of direct animal origin.
- CHO-III-PFM is a protein-free (not chemically-defined) medium without L-glutamine, hypoxanthine or thymidine. It contains plant hydrolysate, and is made without any components of direct animal origin.
- CHO-S-SFM II is a low protein (<100 µg/ml) medium available with or without hypoxanthine and thymidine.

To meet your need for anchorage-dependent systems, we also offer variations: CHO-A-SFM, CHO-III-A-PFM and CD CHO-A Medium. These media do not have surfactant, but may still require additional modification for cell attachment and spreading in some systems.

### Grow CHO Cells to High Density in Serum-free Suspension Culture

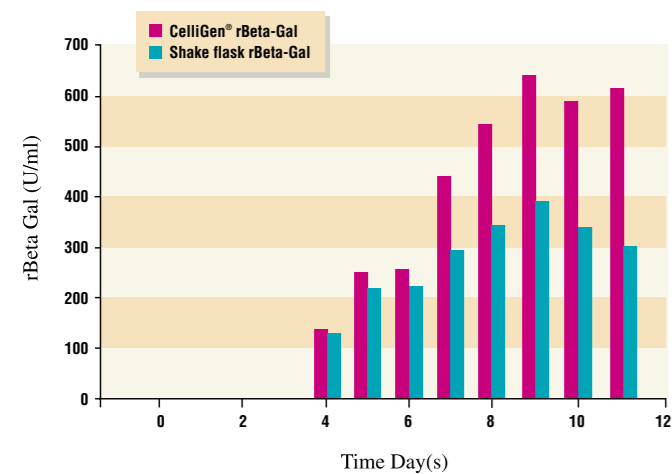


Figure 1. rBeta-galactosidase Expression of rCHO Cells in 5 L CelliGen® Bioreactor

We have developed these media for batch culture systems and can easily modify them for use in fed-batch, continuous-addition or perfusion systems.

Additionally, you can rely on us for custom modifications that include substitution of iron carrier compounds, carbon sources and shear protectants, as well as lipid additives and anti-clumping agents. We also offer wild type CHO cells that have been pre-adapted to suspension culture in CD CHO Medium.

### Cholesterol Lipid Concentrate

As NS0 cells become more popular for large-scale expression of recombinant proteins, removal of serum from the culture system becomes critically important.

We are responding to your requirements for greater levels of media definition and replacement of animal-origin components. GIBCO™ Cholesterol Lipid Concentrate combined with CD Hybridoma Medium is yet another example of our ongoing efforts: a powerful system for growing NS0 cells to high density.

NS0 cells are traditionally grown in either a serum-supplemented medium or a serum-free medium supplemented with an animal-derived lipoprotein supplement. Now, with this new supplement, you can cultivate cholesterol-dependent NS0 cells in a protein-free, chemically-defined environment, achieving sustained, long term growth. (See figure 2.)

Cholesterol Lipid Concentrate is optimized for growth of lipid-dependent hybridoma cell lines and the production of monoclonal antibodies. It is the first of an anticipated series of GIBCO™ cholesterol and plant sterol supplements.

### OPTIMAB® Monoclonal Antibody Production Enhancer

A protein-free, 100X concentrated nutrient supplement that can be used to boost MAb production dramatically, GIBCO™ OptiMAB® includes an alternate carbon source, supplemental amino acids, lipids, and other medium components optimized to ensure maximum MAb yield.

Adding OptiMAB® concentrate to batch hybridoma cultures following achievement of maximum cell density, prior to decrease in cell viability, increases MAb yields by as much as 200% over supplemented cultures. This versatile concentrate is effective with all hybridoma cell lines grown in any serum-free or serum-supplemented medium.

### Improve Product Yields up to 2.5x with Cholesterol Lipid Concentrate and CD Hybridoma Medium

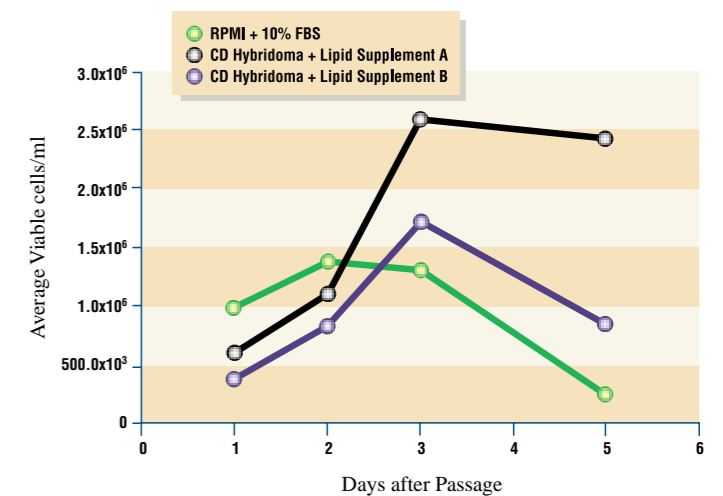
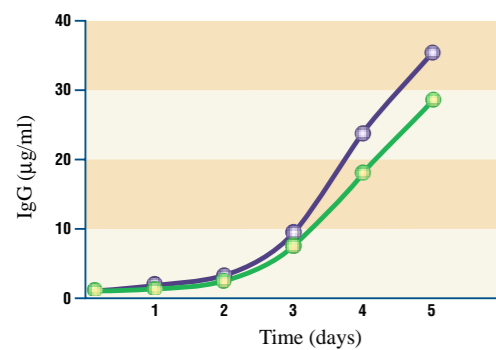
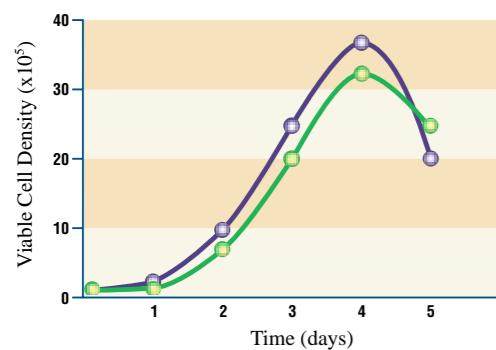


Figure 2. Growth of NS0 Cells in CD Hybridoma Medium Supplemented with Various Lipid Concentrates versus Serum-Supplemented Medium

### Scale Up Successfully with CD Hybridoma Medium



**Growth and IgG Production of Hybridoma TP6-25.3 in a Bioreactor Culture**

**Figure 3.** Cells previously adapted to CD Hybridoma Medium were seeded at  $1 \times 10^5$  viable cells/ml in a bioreactor (●) or a shaker flask control (○). Growth (left panel) and MAb production (right panel) were measured for 5 days.

### CD Hybridoma Medium

In recent years, hybridoma culture media that require serum supplementation have been replaced by a variety of serum-free formulations. However, because many of the original serum-free formulations contain proteins, they no longer are the ideal choice for applications that must meet stringent regulatory guidelines.

Demand for greater levels of media definition, combined with the need to replace components of animal origin with alternatives that perform as well or better, has led us to develop GIBCO™ CD Hybridoma Medium.

- A truly protein-free and chemically-defined medium optimized for hybridoma growth and monoclonal antibody production
- Contains no components of animal or human origin
- Formulated without L-glutamine, providing added stability
- Streamlines purification and downstream processing

Because it is free of animal-derived materials, CD Hybridoma Medium is less likely to contain adventitious agents. Suitable for the culture of recombinant myeloma lines as well as traditional hybridomas, CD Hybridoma Medium outperforms serum-free and serum-supplemented hybridoma media. It is designed for batch systems and can be easily modified for use in other systems. (See figure 3.)

Cells growing in traditional serum-supplemented media can be easily adapted to CD Hybridoma Medium.

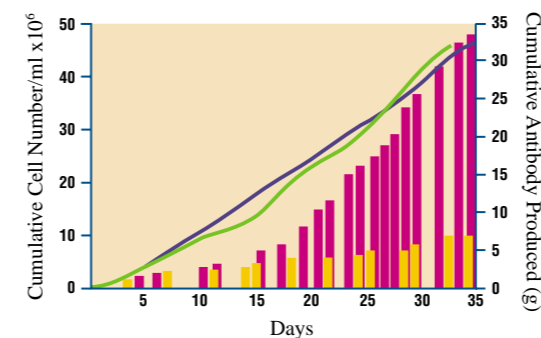
### Hybridoma-SFM

- Complete and ready to use
- Provides excellent growth and maintenance of hybridoma cells
- Offers significantly higher MAb yield and simpler downstream processing than serum-supplemented medium. (See figure 4.)

GIBCO™ Hybridoma-SFM is derived from an optimized serum-free basal formulation supplemented with trace elements, minerals, and a low amount (20 µg/ml) of defined protein (insulin and transferrin). Unlike serum-supplemented media, Hybridoma-SFM is free of BSA, steroids, and endogenous bovine immunoglobulin, facilitating purification of specific MAbs. It is easy to use and supports a wide range of hybridoma cell lines in a variety of culture systems.

Hybridoma-SFM is the medium of choice for growing hybridomas that require insulin or transferrin.

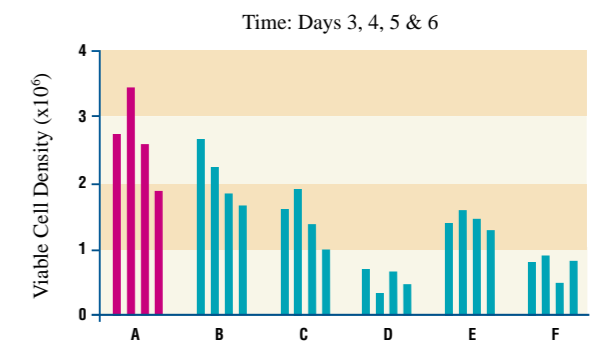
### Optimize Cell Growth with Hybridoma SFM



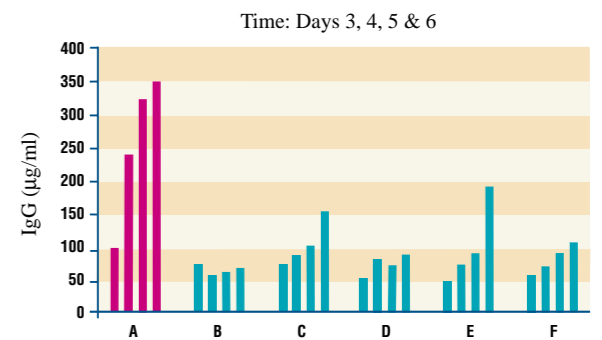
**Cumulative MAb Production and Number of Cells**

**Figure 4.** Cells were cultured in Hybridoma-SFM (■, ■) or D-MEM with FBS (■, ■) (10% until day 10, 7.5% until day 13, and 5% from day 13). Cell number (■, ■) and MAb production (■, ■) were monitored. The culture with Hybridoma-SFM produced significantly more MAb even though the number of cells was similar to serum-supplemented medium.

### Produce Exceptional Yields with CD Hybridoma Medium



**Growth of Hybridoma TP4-3.1**



**IgG Production by Hybridoma TP4-3.1**

**Figure 5.** In comparative cell growth studies, Hybridoma TP4-3.1 (proprietary) cells growing in IMDM + 10% FBS were adapted to growth in other media through a minimum of three passages prior to evaluation of growth and monoclonal antibody production in batch culture. CD Hybridoma Medium was supplemented with 8 mM L-glutamine prior to use. Other media were supplemented as necessary following instructions supplied by the manufacturers. Agitated small-scale suspension cultures of TP4-3.1 cells were grown in 125 mL plastic disposable shake flasks (35 mL culture volume) on an orbital shaker platform at 125–135 rpm. Cells were seeded at  $1 \times 10^5$  viable cells/ml. All cultures were incubated in a humidified atmosphere of 8% CO<sub>2</sub> in air. Total cell counts were determined using an electronic particle counter; viability of cells was estimated by trypan blue dye exclusion. IgG was measured by ELISA.

**KEY:** A-GIBCO™ CD Hybridoma Medium B-GIBCO™ IMDM+10%FBS C-GIBCO™ Hybridoma-SFM D, E and F are media from other manufacturers.

## Get to market faster:

### Call for a seminar.

If you are developing cell culture systems to produce monoclonal antibodies, we invite you to arrange a technical seminar at your site. Our senior scientists will show you how Invitrogen can help you optimize media and culture systems through a highly-customized approach.

We are determined to help you meet your most aggressive timelines with dedicated technical support, providing state-of-the-science expertise through every stage of your development cycle.

### Subscribe to the Biomanufacturing Bulletin.

Keep up with the latest news in bioprocessing from Invitrogen with this informative newsletter.

Read it online at [www.invitrogen.com](http://www.invitrogen.com) or ask for a free subscription.

### Read all about it.

Learn more about our key technologies for antibody production. Comprehensive brochures, catalogs, technical sheets and applications guides are available by mail or in downloadable pdf format online.

## Consult a specialist today.

Let us share our expertise with you. Call your local Invitrogen representative, or e-mail [tech\\_service@invitrogen.com](mailto:tech_service@invitrogen.com) for an in-depth evaluation of how we can work with you to help improve your efficiency and productivity.



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