# Improve Serum-Free Hybridoma Culture with GIBCO® Media

- Choose from chemically-defined, protein-free, and serum-free formulations
- Maximize performance with chemically-defined, protein-free supplements
- Animal-origin-free products minimize risk
- Custom products and packaging available
- Easy to scale up to large volume cultures

	Product	Optimized For	Applications	
ANIMA GREED ORIGIN	CD Hybridoma Medium†	Human, mouse, rat hybridomas, myelomas.	Growth and MAb production. Can be used to express other proteins in engineered	
ANIMA EREE ORIGIN	CD Hybridoma AGT™† Dry granular format of CD Hybridoma Medium.	NS0, NS-1, and other steroid-dependent cells when used with 250X Cholesterol Lipid Concentrate	myeloma cell lines.	
	Hybridoma-SFM† Low-protein 20 μg/ml.	Human, mouse, rat hybridomas, myelomas	Growth and MAb production. Can be used to express other proteins in engineered myeloma cell lines.	
A CREEP ORIGIN	<b>PFHM-II</b> Protein-free Hybridoma Medium.	Human, mouse, rat hybridomas, myelomas	Growth and MAb production. Can be used to express other proteins in engineered myeloma cell lines.	
	Chemically-Defined Media <sup>†</sup> Drug Master File available	Protein-Free Media	m-Free Media Animal-Origin-Free Product	

**Note:** Cell lines from different sources, and different clones of the same cell line, may have highly specific nutritional requirements and may therefore prefer one medium over another. More than one medium formulation (if available) should be evaluated to determine the best option.

### CD Hybridoma Medium

#### Chemically-Defined, Protein-Free Formulation

GIBCO<sup>®</sup> CD Hybridoma Medium is a chemically-defined, protein-free medium optimized for the growth of a variety of human, mouse, and rat hybridomas and myelomas and the production of monoclonal antibodies in stationary or agitated suspension systems. When supplemented with 250X Cholesterol Lipid Concentrate, this medium works well for lipid-dependent or cholesterol-dependent cultures such as NS0-derived lines.

Containing no components of animal origin, CD Hybridoma Medium is also formulated without L-glutamine, providing added stability. Cells growing in traditional serum-supplemented medium can be adapted easily to this medium, typically in as few as 3 passages.

CD Hybridoma Medium streamlines purification and downstream processing. Because it is manufactured with animal-origin-free materials, it 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 serumsupplemented hybridoma media (*figure 1*). It is designed for batch systems but can be modified easily for use in others.

CD Hybridoma Medium is available in a ready-to-use (1X) liquid format and in a new, easy-to-use granular format, Advanced Granulation Technology<sup>™</sup> (AGT<sup>™</sup>). AGT<sup>™</sup> Media are complete, pH pre-adjusted, and require only standard supplementation with L-glutamine (or GlutaMAX<sup>™</sup>-I Supplement) for L-glutamine-dependent systems. The granules dissolve instantly for faster media preparation times. AGT<sup>™</sup> media can help to reduce total cycle costs, decreasing time involved in raw material planning, procurement, and testing, as well as media preparation.

# Serum-Free Media

GIBCO<sup>®</sup> Serum-Free Media do not require supplementation with serum, but may contain discrete proteins or bulk protein fractions.

# Protein-Free Media

GIBCO<sup>®</sup> Protein-Free Media contain no proteins, but may contain plant or yeast hydrolysates. Many are animal-origin-free.

# **Chemically-Defined Media**

GIBCO<sup>®</sup> Chemically-Defined Media contain no proteins, hydrolysates, or components of unknown composition. These media are animal-origin-free and all components have a known chemical structure.

- Completely defined system
   eliminates variability
- Consistent performance improves reproducibility
- Decrease possibility of contamination by adventitious agents
- Save time with simplified purification and downstream processing



#### IgG Production by Hybridoma TP4-3.1



Figure 1. 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 × 10<sup>5</sup> viable cells/ml. All cultures were incubated in a humidified atmosphere of 8% CO<sub>2</sub> in air. Total cell 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.

#### Growth of Hybridoma TP4-3.1

## 250X Cholesterol Lipid Concentrate

#### **Chemically-Defined Formulation**

Used to supplement GIBCO<sup>®</sup> media such as CD Hybridoma Medium, animal-origin free 250X Cholesterol Lipid Concentrate allows cultivation of NS0, NS-1, and related sterol-dependent cell lines in a protein-free and chemically-defined environment, supporting growth and expression while simplifying downstream purification. The supplement permits filtration of complete 1X medium with minimal loss of lipids.

#### **PFHM-II Protein-Free Hybridoma Medium**

#### **Protein-Free Formulation**

PFHM-II is a protein-free medium optimized for the growth of a variety of human, mouse, and rat hybridomas and myelomas and the production of monoclonal antibodies. It contains no polypeptides such as growth or attachment factors which could interfere with downstream protein purification. PFHM-II performs as well as serum-supplemented media for MAb production.

#### Hybridoma-SFM

#### Serum-Free Formulation

Hybridoma-SFM is a very low-protein medium (20 µg/ml) optimized for the growth of a variety of human, mouse, and rat hybridomas and myelomas and the production of monoclonal antibodies. It provides excellent growth and maintenance of hybridoma cells and offers significantly higher MAb yields and simpler downstream processing than serum-supplemented media (*figure 2*). A complete, ready-to-use medium, it is 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.

Most hybridomas require only minor sequential adaptation or no adaptation to this medium. Its very low protein content facilitates monoclonal antibody purification.

# OptiMAb<sup>®</sup> Monoclonal Antibody Production Enhancer

A protein-free, 100X-concentrated nutrient supplement that can be used to boost MAb production dramatically, OptiMAb<sup>®</sup> Enhancer includes an alternate carbon source, supplemental amino acids, lipids, and other media components optimized to ensure maximum MAb yield.

Adding OptiMAb<sup>®</sup> concentrate to batch hybridoma cultures following achievement of maximum cell density, prior to decrease in cell viability, has been shown to increase MAb yields by as much as 200% over supplemented cultures. This versatile concentrate is effective with hybridoma cell lines grown in any serum-free or serum-supplemented medium.

#### **Cumulative MAb Production and Number of Cells**





Figure 2. 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 MAbs even though the number of cells was similar to serum-supplemented medium.

# **Custom Production and Packaging**

When you need a unique formulation or special packaging, our Custom Product Services team can modify GIBCO<sup>®</sup> catalog media formulations and packaging to meet your particular requirements.

Media are available in different formats for easy scale-up to meet the needs of various levels of product development: R&D, process development, pilot plant, and manufacturing.

We can produce volumes as small as a few liters to > 30,000 liters, or > 100,000 liters in dry format. In addition, we offer large media bag packaging options up to 500 liters.

The Custom Product Services team can also assess feasibility and provide options for formulation design, testing, and packaging for your proprietary formulations. **For information call 1-800-955-6288, Ext. 46966.** 

### References

#### CD Hybridoma Medium

Csirke, B., Godwin, G., and Gorfien, S. (1999) *Focus®* **21**, 33. Gorfien, S., Godwin, G., Samrock, R., and Tilkins, M. L. (1999) *Focus®* **21**, 75.

#### Hybridoma-SFM

Mullins T.D., Dzimian, J.L., Fike, R.M., and Weiss, S.A. (1991) Focus® 13, 91.

#### PFHM-II

Fike, R.M., Pfohl, J., Jayme, D., and Weiss, S.A. (1990) Focus® 12, 79.

#### Ordering Information

Description	Catalog No.	Size
CD Hybridoma Medium (1X), liquid	11279-015 11279-023	500 ml 1,000 ml
CD Hybridoma AGT™ Dry granular format of CD Hybridoma Medium.	12372-025 12372-017	1 × 1 L 1 × 10 L
PFHM-II (1X), liquid	12040-077	1,000 ml
Hybridoma-SFM (1X), liquid	12045-084 12045-076	500 ml 1,000 ml
OptiMAb® Monoclonal Antibody Production Enhancer (100X), liquid	11910-031	100 ml
250X Cholesterol Lipid Concentrate	12531-018	100 ml
Related Products		
Nutritional Supplements		
GlutaMAX <sup>™</sup> -I Supplement Stable form of L-glutamine.	35050-061	100 ml
L-Glutamine-200 mM (100X), liquid	25030-081	100 ml
Chemically-Defined Lipid Concentrate Lipid emulsion to reduce or replace FBS in hybridoma cultures.	11905-031	100 ml
Hybridoma Reagents		
HAT Supplement (50X), liquid	21060-017	100 ml
HT Supplement (100X), liquid	11067-030	50 ml



www.invitrogen.com

Corporate Headquarters: Invitrogen Corporation • 1600 Faraday Avenue • Carlsbad, California 92008 U.S.A. Tel: 1 760 603 7200 • Tel (Toll Free): 1 800 955 6288 • Toll Free Fax: 1 800 331 2286 • E-mail: tech\_service@invitrogen.com European Headquarters: Invitrogen Ltd • 3 Fountain Drive • Inchinnan Business Park • Paisley PA4 9RF, UK Tel: + 44(0) 141 814 6100 • Fax: + 44(0) 141 814 6260 • E-mail: eurotech@invitrogen.com



These products are for research use, and where appropriate, as raw material components in further cell culture manufacturing applications. They are not intended for - human or animal diagnostic, therapeutic, or other clinical uses, unless otherwise stated. © 2003 Invitrogen Corporation PAS03-050MS Part No. 332-032443B

