

## Serum-Free and Protein-Free Media for Hybridoma Culture

### Hybridoma-SFM

Serum-free, low protein medium for hybridoma growth and monoclonal antibody production.

**CAUTION:** Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB<sub>s</sub>Ag. Handle in accordance with established bio-safety practices

Cat. No. 12045-084 500 mL  
12045-076 1000 mL

With L-glutamine  
Storage Conditions: 2 to 8°C, in the dark.

### PFHM II

Protein-free Hybridoma Medium

Cat. No. 12040-077 1000 mL

With L-glutamine  
Storage conditions: 2 to 8°C, in the dark.

### Intended Use

GIBCO™ products for hybridoma culture have been designed and optimized for the serum-free growth of a variety of hybridoma cell lines and production of monoclonal antibodies.

### Introduction

Traditional hybridoma culture media requiring serum supplementation have in recent years been replaced by a variety of commercially available serum-free formulations. Many serum-free formulations contain proteins (e.g., insulin, transferrin, albumin) and/or protein hydrolysates and lysates. As a result of a trend towards greater levels of media definition and the need for replacement of components of animal origin with non-animal derived materials, many serum-free media formulations are considered unacceptable for certain applications.

Hybridoma-SFM and PFHM II represent a spectrum of serum-free products for growth of hybridomas and monoclonal antibody production. **Hybridoma-SFM** is a serum-free, low protein (20 µg/mL protein as insulin and transferrin) medium that supports growth and monoclonal antibody production of a variety of hybridoma cell lines. **PFHM II** is a protein-free, chemically defined medium that is intended as a monoclonal antibody production medium, although it also has been used to grow a variety of hybridoma cell lines.

### Features

	PFHM II	Hybridoma-SFM
Protein-Free	X	
Contains insulin and transferrin		X
Contains Phenol red	X	X
Contains surfactant*		X
Contains inorganic iron carrier**	X	

### Precautions

\* PFHM II does not contain a surfactant. If used for agitated suspension culture, supplement with 0.1% PLURONIC® F-68

\*\* Medium should be pre-screened to determine potential interference of inorganic iron carrier(s) with antibody detection and/or purification method.

Hybridoma-SFM and PFHM II work well for a variety of hybridoma systems, but will not grow cholesterol dependent cell lines (e.g., NS0 and derivatives) without further supplementation. Supplementation with a lipoprotein preparation or other source of cholesterol will be required for cholesterol dependent cell lines.

Addition of antibiotics should not be used as a substitute for proper sterile technique. In most instances, antibiotics are neither necessary nor advised. However, in those instances where antibiotics are desired, most general antibiotics are compatible with PFHM II including penicillin/streptomycin, gentamicin, anti-PPLO, linocin and Fungizone®. **It is important not to use the following: kanamycin sulfates, neomycin sulfates or penicillin/streptomycin/neomycin mixtures.**

### Instructions For Use

#### Physical Conditions

37°C ± 0.5°C in a humidified atmosphere of 5 - 10% CO<sub>2</sub> in air. Caps of flasks should be loosened to permit gas exchange. Cultures may be grown in stationary suspension culture (e.g., T-flask) or in agitated suspension culture (shaker or spinner flasks). Adequate headspace should be provided to facilitate gas exchange. (e.g., for a 125 mL shaker flask, use no more than 35 mL culture volume). Shaker flasks should be rotated at 125 - 135 rpm; agitation speed in spinner flasks will depend upon the impeller design. Protect cultures from light.

#### Adaptation of Cells to Serum-free or Protein-free Media

A sequential adaptation protocol may be necessary if direct adaptation does not work. In both cases, the cells should be in mid-logarithmic growth phase with high (>90%) viability. Success of the adaptation method will depend upon the particular hybridoma cell line and the culture conditions employed. It is recommended that backup cultures in the original medium be maintained until success with the new medium has been achieved.

- A. Direct Adaptation
  1. Transfer hybridoma cells growing in serum supplemented medium to serum-free medium which has been prewarmed to 37°C. Seeding density should be double the normal seeding density for the cell line. Incubate the cells at 37°C in a humidified atmosphere of 5-10% CO<sub>2</sub> in air.
  2. Monitor cell growth until viable cell density reaches 1 × 10<sup>6</sup>/mL. Subculture the cells to a viable cell density of 1-2 × 10<sup>5</sup>/mL in fresh serum-free medium. Subculture in this manner, monitoring cell growth and viability, for 3 to 5 passages.
  3. If the culture fails to maintain acceptable growth and viability over 3-5 passages during direct adaptation, use the sequential adaptation method.
- B. Sequential Adaptation
  1. Inoculate hybridoma cells at double the normal seeding density in a 75:25 (v/v) mixture of serum supplemented : serum-free medium.
  2. Monitor the culture until the density reaches 1 × 10<sup>6</sup> viable cells/mL. Then subculture into a 50:50 (v/v) mixture of serum supplemented : serum-free medium.
  3. Monitor the culture until the density reaches 1 × 10<sup>6</sup> viable cells/mL. Then subculture into a 25:75 (v/v) mixture of serum supplemented : serum-free medium.
  4. Monitor the culture until the density reaches 1 × 10<sup>6</sup> viable cells/mL. Then subculture into 100% serum-free medium.
  5. **NOTE that it may be necessary to subculture more than once into a given mixture of serum supplemented : serum-free medium until the cells become acclimated. It is advisable to keep a backup culture in the previous media mixture until the cells have adapted.**

### Cryopreservation

1. Prepare desired quantity of cells, harvesting in mid-log phase of growth with viability > 90%.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium (50% fresh medium : 50% conditioned medium<sup>1</sup> + DMSO to a final concentration of 7.5%) to give a final cell density of 0.5 - 1.0 × 10<sup>7</sup> cells/mL.
3. Prepare the required volume of cryopreservation medium and hold the medium at 4°C until use (make cryopreservation medium on day of intended use).
4. Pellet the cells from culture medium at 100 × g for 5 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (e.g., 4.5 mL in a 5.0 mL vial).
6. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Frozen cells are stable indefinitely under liquid nitrogen.

<sup>1</sup> Note that conditioned medium should be obtained from a high viability, mid-log culture of cells.

### Recovery from Cryopreservation

1. Recover cultures from frozen storage by rapid thawing of a vial of cells in a 37°C water bath with shaking just until the medium thaws.
2. Transfer the entire contents of the vial into the appropriately sized vessel so that the cells are seeded at 5 × 10<sup>5</sup> cells/mL of complete growth medium.
3. Incubate the culture in a humidified atmosphere of 5-10% CO<sub>2</sub> in air at 37±0.5°C. **Do not centrifuge the cells as they are extremely fragile upon recovery from cryopreservation.**
4. Maintain the culture between 5 × 10<sup>4</sup> and 10 × 10<sup>5</sup> viable cells/mL for the first two subcultures following recovery; thereafter, returning to the normal maintenance schedule.

### Quality Control

GIBCO specialty media for hybridoma applications are performance tested using either a myeloma or hybridoma cell line. Additional standard evaluations are pH, osmolality and tests for the absence of bacterial and fungal contaminants.

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Fungizone®. Is a registered trademark of E.R. Squibb & Sons

For further information on this or other GIBCO™ products, contact Technical Services at the following:

United States TECH-LINE<sup>SM</sup> : 1 800 955 6288  
Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at [www.invitrogen.com](http://www.invitrogen.com).

**For research use only.**

**CAUTION: Not intended for human or animal diagnostic or therapeutic uses.**

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