gibco by life technologies™

Hybridoma-Serum Free Media (SFM)

PFHM-II

Description

Hybridoma-SFM and PFHM-II are serum-free products optimized for growth of hybridomas and monoclonal antibody production. Hybridoma-SFM is a very low (<20 µg/mL) protein medium. The very low protein content facilitates monoclonal antibody purification.

PFHM-II is a protein-free, ready-to-use medium that contains no polypeptide growth or attachment factors, or mediators that may complicate downstream processing and final product purification. PFHM-II also performs well as serum-supplemented media for MAb production and also may be used as a growth medium.

Product	Catalog No.	Amount	Storage	Shelf Life*
Hybridoma-SFM, with L-glutamine	12045-084 12045-076	500 mL 1000 mL	2°C to 8°C; Protect from light 2°C to 8°C; Protect from light	12 months 12 months
Hybridoma-SFM (1X), powder w/ L-glutamine	12300-067	1 × 10 L	2°C to 8°C; Store Dark and Dry	33 months
PFHM-II, with L-glutamine	12040-077	1000 mL	2°C to 8°C; Protect from light	12 months
PFHM-II (1X), powder w/ L-glutamine	23600-042	1 × 10 L	2°C to 8°C; Store Dark and Dry	24 months

* Shelf Life duration is determined from Date of Manufacture.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Product Use (Cat. no. 12040 Only)

Caution: For manufacturing, processing, or repacking.

Important Information

- Hybridoma-SFM and PFHM-II require supplementation with a cholesterol supplement or some other source of cholesterol (i.e., 250X Cholesterol Lipid Concentrate) for growth of cholesterol-dependent cell lines (e.g., NS0 and derivatives).

	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	

* 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.

- In most instances, antibiotics are neither necessary nor advised. However, where antibiotics are required, most general antibiotics are compatible with PFHM-II, including penicillin/streptomycin, gentamicin, anti-PPLO, linocin, and Fungizone™ Reagent. **Do not use** kanamycin sulfates or neomycin sulfates.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ **CAUTION!** Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB_sAg. Handle in accordance with established bio-safety practices.

Reconstitute Powdered Media

1. Add powdered media to 9 L room temperature deionized or distilled water. Rinse package to remove all traces of powder.

Hybridoma-SFM

2. Add 2.45 g $NaHCO_3$ per L of medium. Stir until completely dissolved, **do not** heat.
3. Adjust to pH 8.0 with 1N NaOH while stirring. Slowly bring the pH to 7.0–7.1 using 1N HCl (Filtration will raise the pH 0.1–0.3 units; final post-filtration pH should be 7.2–7.4).
4. Add distilled water to final volume of 10 L. Check pH and osmolality (Osmolality should be 320–345 mOsm/kg).

PFHM-II

2. Adjust pH to 7.0 using either 1N HCl or 1N NaOH while stirring.
3. Add 2.0 g $NaHCO_3$ per L of medium. Stir until completely dissolved, **do not** heat.
4. Readjust pH to 7.0, (see step 2 PFHM-II). Add distilled water to final volume of 10 L.

Filter Sterilize

5. Stir for 15–20 minutes at room temperature. Filter sterilize using a surfactant-free 0.2 µm filter and dispense into sterile, clean containers. Protect from light.

Prepare Media

Hybridoma-SFM and PFHM-II do not require supplementation except as noted for cholesterol dependent cell lines, agitated suspension cultures using PFHM-II, and if antibiotics are desired.

1. Aseptically add 2 mL of 250X Cholesterol Lipid Concentrate to 500 mL of Hybridoma-SFM or PFHM-II Medium.
2. Aseptically add antibiotics, if required.

Culture Conditions

Media: Hybridoma-SFM or PFHM-II Medium
Cell Type: Hybridoma
Culture Type: Suspension
Culture Vessels: Shake flasks, roller bottles or bioreactor.
Temperature Range: 36°C to 38°C
Incubator Atmosphere: Humidified atmosphere of 5–10% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a tissue culture flask containing 30 mL prewarmed Hybridoma-SFM or PFHM-II without antibiotics.
3. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.
4. Subculture cells 3–5 days post thaw.

Adapt Hybridoma Cells to Hybridoma-SFM or PFHM-II

Successful adaptation will depend upon the particular hybridoma cell line and the culture conditions employed. We recommended that backup cultures in the original medium be maintained until success with the new medium has been achieved.

Note: It is critical that cell viability be at least 90% and cells be in the mid-logarithmic phase of growth prior to adaptation.

Direct Adaptation

1. Subculture hybridoma cells grown in conventional medium with 5–10% serum or other serum-free medium into prewarmed Hybridoma-SFM or PFHM-II. During the adaptation procedure seeding density should be double the normal seeding density for the cell line.
2. Monitor cell growth using Countess® Automated Cell Counter (or alternate suitable method) until the viable cell density reaches 1×10^6 viable cells/mL. Subculture the cells to a viable cell density of $1–2 \times 10^5$ viable cells/mL in fresh prewarmed Hybridoma-SFM or PFHM-II.
3. Continue to monitor and passage cells for 3–5 passages until consistent growth is achieved.

Note: If suboptimal performance is observed over 3–5 passages using the direct adaptation method, use the sequential adaptation method.

Sequential Adaptation

1. Subculture Hybridoma cells grown in conventional medium with 5-10% serum or other serum-free medium into a 25:75 ratio of fresh Hybridoma-SFM or PFHM-II to the original media. During the adaptation procedure seed at double the normal seeding density.
2. Monitor cell growth until the viable cell density reaches 1×10^6 viable cells/mL. Subculture cells (dilute to $1–2 \times 10^5$ viable cells/mL) into stepwise increasing ratios of fresh PFHM-II or Hybridoma-SFM to original medium with each subsequent passage (50:50, 75:25, 90:10 followed by 100% Hybridoma-SFM or PFHM-II). Multiple passages at each step may be required.
3. Continue to monitor and passage cells until consistent growth is achieved. After several passages of consistent growth and viability in 100% complete Hybridoma-SFM or PFHM-II the culture is considered to be adapted.

Cryopreservation

1. Prepare the desired quantity of cells in a tissue culture flask, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of $0.5–1 \times 10^7$ cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% medium (50:50 ratio of fresh to conditioned media) +7.5% DMSO on the day of intended use. Filter sterilize and store at 4°C until use. **Important:** Conditioned medium should be obtained from a high viability, mid-log culture of cells.
4. Harvest cells by centrifugation at 100 × g for 5–10 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 (i.e., 1 mL in a 2-mL cryovial).
6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen (vapor phase); storage at –200°C to –125°C is recommended.

Related Products

Product	Catalog No.
250X Cholesterol Lipid Concentrate	12531
L-Glutamine, 200 mM (100X), Liquid	25030
GlutaMAX™-I, 200 mM (100X), Liquid	35050
Water, Distilled	15230
Gibco® Water for Injection (WFI) for Cell Culture	A12873
Pluronic® F-68, 10% (100X)	24040
Penicillin-Streptomycin, Liquid	15140
Gentamicin Reagent Solution (50 mg/mL), Liquid	15750
Antibiotic-Antimycotic (100X), Liquid	15240
Fungizone® Antimycotic, Liquid	15290
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Temperature Limitation	Manufacturer	Batch code	Use By:	Catalog number
				
Caution, consult accompanying documents	Consult instructions for use	Keep away from light	Sterilized using aseptic processing techniques	

Limited Use Label License: Internal Research and Bioproduction Use

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) to culture cells for the purpose of producing a product wherein the product will be used for any or all of the following: (i) internal research use by the purchaser; (ii) resale for internal research use by third parties; (iii) performance of research conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties; (iv) resale for use as a human therapeutic agent or diagnostics product or component by third parties; (v) performance of manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties.

The purchase of this product does not grant the purchaser any additional rights, including (without limitation) the right to transfer or resell the product in any form, the right to use the product as a therapeutic agent or diagnostics test component, or to use the product to perform other tests on a contract or fee per test basis for or on behalf of third parties. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Product Warranty

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**