Protein-Free Hybridoma Medium (PFHM-II)

Catalog Number 12040077

Pub. No. MAN0026551 Rev. 1.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

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

Product description

The Gibco™ Protein-Free Hybridoma Medium (PFHM-II) is a serum-free product optimized for growth of hybridomas and monoclonal antibody production. 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 monoclonal antibody production and also may be used as a growth medium.

Contents and storage

Product	Cat. No.	Amount	Storage	Shelf Life[1]
PFHM-II	12040077	1000 mL	2–8°C; Protect from light	12 months

[1] Shelf Life duration is determined from Date of Manufacture.

Culture conditions

Media: PFHM-II

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.

Procedural guidelines

IMPORTANT!

- PFHM-II requires supplementation with a cholesterol supplement or some other source of cholesterol (i.e., Cholesterol Lipid Concentrate (250X)) for growth of cholesterol-dependent cell lines (e.g., NS0 and derivatives).
- PFHM-II is protein-free and contains phenol red.
- PFHM-II contains an inorganic iron carrier.

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

Prepare media

- PFHM-II does not require supplementation except as noted for cholesterol dependent cell lines, agitated suspension cultures using PFHM-II, and if antibiotics are desired.
- Agitated PFHM-II does not contain surfactant. If used for agitated suspension culture, supplement with 0.1% Pluronic™ F-68.

1. Aseptically add 2 mL of Cholesterol Lipid Concentrate (250X) to 500 mL of PFHM-II.
2. Aseptically add antibiotics, if required.

   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 Amphotericin B.

   Do not use kanamycin sulfates or neomycin sulfates.

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 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 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 adaption

1. Subculture hybridoma cells grown in conventional medium with 5–10% serum or other serum-free medium into prewarmed 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™ 3 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 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 adaption

1. Subculture hybridoma cells grown in conventional medium with 5–10% serum or other serum-free medium into a 25:75 ratio of fresh 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 to original medium with each subsequent passage (50:50, 75:25, 90:10 followed by 100% 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 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

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
Cholesterol Lipid Concentrate (250X)	12531018
L-Glutamine (200 mM)	25030024
GlutaMAX™ I Supplement	35050061
Water, Distilled	15230170
Water For Injection (WFI) for Cell Culture	A1287301
Pluronic™ F-68	24040032
Penicillin-Streptomycin	15140122
Gentamicin (50 mg/mL)	15750060
Antibiotic-Antimycotic (100X)	15240096
Amphotericin B	15290018
Trypan Blue Solution, 0.4%	15250061
Countess™ 3 Automated Cell Counter Starter Package + REX Extended Warranty	A250298

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
1.0	15 April 2022	New user guide for Cat. No. 12040077 to align with Use statement.