ExpiSf™ CD Medium

Catalog Numbers A3767801, A3767802, A3767803, A3767804, A3767805

Pub. No. MAN0017561 **Rev.** 2.0

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**.

Product description

Gibco™ ExpiSf™ CD Medium is a chemically defined, yeastolate-free, serum-free, protein-free, animal origin-free medium developed specifically for the high-density growth of ExpiSf9™ cells for expression of recombinant proteins in suspension culture using the Baculovirus Expression Vector System (BEVS). The medium is designed for scalable transfection and baculovirus production, and does not interfere with nor reduce the activity of ExpiFectamine™ Sf Transfection Reagent. Baculovirus-based protein expression can also be performed directly in ExpiSf™ CD Medium. As a complete, ready-to-use medium, ExpiSf™ CD Medium requires no additional supplementation.

Contents and storage

Contents	Cat. No.	Amount	Storage
ExpiSf™ CD Medium	A3767801	500 mL	2°C to 8°C. Protect from light.
	A3767802	1 L	
	A3767803	6 × 1 L	
	A3767804	10 L	
	A3767805	20 L	

Required materials not supplied

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

Item	Source
ExpiSf9™ Cells	A35243
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	4115-0125

Procedural guidelines

- ExpiSf™ CD Medium is sensitive to light. For optimal results, store media protected from light.
- ExpiSf™ CD Medium is a complete, ready to use medium. Do not add L-Glutamine or surfactant such as Pluronic™ F-68.
- Antibiotics are not recommended.

Culture conditions

Media: ExpiSf™ CD Medium

Cell line: ExpiSf9™ cells

Culture type: Suspension

Shake flask type: It is recommended to use PETG, non-baffled, vented Erlenmeyer flasks; however, baffled Erlenmeyer flasks may also be used.

Temperature range: 27.5°C ±0.5°C

Shaker speed: For shakers with a 19-mm or 25-mm shaking diameter, set the shake speed to 125 ±5 rpm. For shakers with a 50-mm shaking diameter, set the shake speed to 95 ±5 rpm.

Incubator type: Non-humidified, air regulated, non-CO_2 atmosphere. Ensure proper gas exchange and minimize exposure of culture to light.

Thaw ExpiSf9™ cells

1. Remove one vial of cells from liquid nitrogen and swirl in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly until only a small amount of ice remains.

   Note: Do not submerge the vial in the water.

2. Just before the cells are completely thawed, decontaminate the vial by wiping with 70% ethanol before opening it in a laminar flow hood.

3. Transfer the entire contents of the cryovial with a 2-mL or 5-mL pipette into a 125-mL PETG, sterile, non-baffled, vented shake flask containing 25 mL of pre-warmed (i.e., room temperature) ExpiSf™ CD Medium.

   IMPORTANT! ExpiSf™ CD Medium comes in a ready-to-use format. For suspension growth, baculovirus production, and protein expression applications, use the ExpiSf™ CD Medium without any supplementation.

4. Incubate the cells at 27.5°C ±0.5°C in a non-humidified, non-CO_2 atmosphere incubator on an orbital shaker platform set at 125 ±5 rpm (for shakers with a 19-mm or 25-mm shaking diameter) or 95 ±5 rpm (for shakers with a 50-mm shaking diameter).

5. Three days post-thaw, determine viable cell density and percent viability.

   Note: Cell viability should be ≥80% by three days post-thaw.

6. Continue to monitor cell density and viability and subculture the cells once the culture has reached $5 \times 10^6 – 10 \times 10^6$ viable cells/mL (typically 4–5 days post-thaw).

Guidelines to subculture ExpiSf9™ cells

- Passage ExpiSf9™ cells directly in ExpiSf™ CD Medium.
- It is recommended to use a 125-mL or 250-mL PETG, sterile, non-baffled, vented shake flask containing 20–32% total working volume of cell suspension. When using larger flasks, the total working volume should be between 25–33%.
- Subculture ExpiSf9™ cells when they attain a density of $5 \times 10^6–10 \times 10^6$ viable cells/mL.
- Cells should exhibit only minimal clumping during routine cell culture maintenance.

Subculture ExpiSf9™ cells

1. Using the viable cell density, calculate the volume of cell suspension required to seed a new shake flask according to the recommended seeding densities in Table 1 and the recommended culture volumes in Table 2.

2. Transfer the calculated volume of cells to fresh, pre-warmed (i.e., room temperature) ExpiSf™ CD Medium in a shake flask.

3. Incubate flasks at 27.5°C ±0.5°C in a non-humidified, non-CO_2 atmosphere incubator on a shaker platform set at 125 ±5 rpm (for shakers with a 19-mm or 25-mm shaking diameter) or 95 ±5 rpm (for shakers with a 50-mm shaking diameter) until cultures reach a density of $5 \times 10^6–10 \times 10^6$ viable cells/mL.

   Note: If necessary, modify the initial seeding density to attain the target cell density of $5 \times 10^6–10 \times 10^6$ viable cells/mL at the time of subculturing.

4. Repeat Steps 1–3 to maintain or expand the cells for baculovirus production or protein expression.

Table 1 Recommended seeding densities for routine cell culture

Subculture timing	Seeding density
Cells ready 3 days post-passaging	$0.7 \times 10^6–1.0 \times 10^6$ viable cells/mL
Cells ready 4 days post-passaging	$0.4 \times 10^6–0.6 \times 10^6$ viable cells/mL

Table 2 Recommended culture volume using different non-baffled shake flask sizes

Shake flask size	Recommended culture volume
125-mL	25–30 mL
250-mL	50–60 mL
500-mL	100–120 mL
1-L	200–240 mL
2-L	400–480 mL
3-L	600–800 mL

Guidelines to cryopreserve ExpiSf9™ cells

- ExpiSf9™ cells can be frozen directly in ExpiSf™ CD Medium.
- Freeze ExpiSf9™ cells at a final density of 1×10^7 viable cells/mL in 1.5 mL total volume of 92.5% conditioned ExpiSf™ CD Medium and 7.5% DMSO.
- Allow cells to attain a viable cell density of 3×10^6– 4.5×10^6 cells/mL and ≥95% viability before harvest.

  Note: For cryopreservation, the viable cell density at time of harvest is critical for optimal cell health. Therefore, make sure to only harvest cells when they are within the recommended 3×10^6– 4.5×10^6 viable cells/mL range. If viable cell density is too low at the time of harvest, return cells to the incubator until the cells reach the recommended density. If viable cell density is too high at the time of harvest, subculture the cells at 0.5×10^6– 0.6×10^6 cells/mL and prepare for harvest again after 3–4 days.

Cryopreserve ExpiSf9™ cells

1. Centrifuge the cells at 300 × *g* for 5 minutes.
2. Decant the spent "conditioned" medium into a sterile conical tube or bottle.

   IMPORTANT! Do not discard the supernatant.

3. Resuspend the cell pellet using the appropriate volume of conditioned medium collected above to achieve a final cell density of 1×10^7 viable cells/mL, and gently resuspend the cell pellet by pipetting up/down.
4. Add the required volume of DMSO (7.5% final) to the cell suspension and gently mix.
5. Immediately aliquot 1.5 mL cell suspension volume per cryovial.
6. Freeze the cells in an automated or manual controlled-rate freezing apparatus following standard procedures.

   Note: For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.

7. Transfer frozen vials to liquid nitrogen (vapor phase) for long-term storage.

Guidelines to adapt Sf9 and Sf21 cells to ExpiSf™ CD Medium

- You may adapt your Sf9 and Sf21 cells for growth in ExpiSf™ CD Medium.
- We recommend using a sequential adaptation protocol.
- It is critical that cell viability be at least 90% and the growth rate be in mid-logarithmic phase prior to initiating adaptation procedures.

Note: This procedure is meant to serve as a guideline for your adaptation process. Different growth kinetics and maximum cell density may be observed during the adaptation of your Sf9 or Sf21 cell line.

Adapt Sf9 and Sf21 cells to ExpiSf™ CD Medium

1. Subculture Sf9 or Sf21 cells into a 25:75 ratio of ExpiSf™ CD Medium to the original media.

   Note: In the event that cells lag during this first transition, it is possible to reduce the ratio to 10:90 as a first step and/or to use conditioned media for this first step.

   Note: During the adaptation procedure use a seeding density of 1.0×10^6 viable cells/mL at each sub-culturing step.

2. Incubate at 27.5°C ±0.5°C in a non-humidified, non-CO_2 atmosphere incubator on an orbital shaker platform set at 125 ± 5 rpm (19-mm or 25-mm shaking diameter) or 95 ± 5 rpm (50-mm shaking diameter).
3. Three to four days after subculture, remove a small amount of the cell suspension and perform a cell count to determine viable cell density and viability.

   Note: If cells are >90% viable and $\geq 5 \times 10^6$ viable cells/mL, they are ready to be passaged. If cells are at ≥90% viability, but have not reached the desired density, return cells to the incubator for 1–2 days, until the viable cell density is $\geq 5 \times 10^6$ cells/mL.

4. Subculture when the viable cell density is $\geq 5 \times 10^6$ cells/mL by passaging cells into a 50:50 ratio of ExpiSf™ CD Medium to original medium.
5. Repeat Step 4, increasing stepwise the ratio of ExpiSf™ CD Medium to original medium (75:25 followed by 90:10) until the cells are transferred into 100% ExpiSf™ CD Medium.

   Note: Multiple passages at each step may be needed. Subculture cells when viable cell density is $\geq 5 \times 10^6$ cells/mL at each step.

6. Once fully adapted to ExpiSf™ CD Medium, the viable cell density should exceed 5×10^6 cells/mL with a viability ≥90% within 3–4 days of subculture (when using a seeding density of 1×10^6 cells/mL).

   At this stage the seeding density may be reduced to 0.5×10^6– 1.0×10^6 viable cells/mL for subsequent passaging.

7. At this point, it is recommended to create a cell bank of the adapted cells. As with any cell line adaptation, it is best practice to continue to culture and monitor the cells as they gain more passages in ExpiSf™ CD Medium and generate additional cell banks, as necessary.

Related products

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

Item	Source
ExpiSf™ Expression System Starter Kit	A38841[1] A39112[2] A39111[3]
ExpiFectamine™ Sf Transfection Reagent	A38915
ExpiSf™ Protein Production Kit, 1 L	A3767806
ExpiSf™ Protein Production Kit, 10 L	A3767807
ExpiSf™ Protein Production Kit, 5 × 10 L	A3767808
Bac-to-Bac™ Baculovirus Expression System	10359-016
Bac-to-Bac™ C-His TOPO™ Expression System	A11100
Bac-to-Bac™ N-His TOPO™ Expression System	A11101
Bac-to-Bac™ HBM TOPO™ Secreted Expression System	A11339
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	4115-0125
Trypan Blue Stain	15250

[1] North America, Europe
[2] Latin America, Asia Pacific, Japan
[3] China

Limited product warranty

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