ExpiFectamine™ Sf Transfection Reagent

Catalog Number A38915

Pub. No. MAN0017502 **Rev.** A.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

ExpiFectamine™ Sf Transfection Reagent is a proprietary, cationic lipid formulation suitable for transfecting DNA into insect cells. This reagent is a component of the ExpiSf™ Expression System and has been optimized to transfect ExpiSf9™ cells using the Bac-to-Bac™ Baculovirus Expression System.

Contents and storage

Product	Cat. No.	Contents	Storage
ExpiFectamine™ Sf Transfection Reagent	A38915	1 mL	Store at 4°C. Do not freeze

Required materials not supplied

Item	Source
ExpiSf9™ Cells	A35243
ExpiSf™ CD Medium	A3767801-A3767805
Opti-MEM™ I Reduced Serum Medium	31985062
Bacmid DNA prepared using the Bac-to-Bac™ Baculovirus Expression System	10360014, A11098, A11099, A11338
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	4115-0125
Thomson Instrument Company 24-well deep-well plate	NC0012954
Non-humidified, air-regulated, non-CO_2 atmosphere incubator set at 27°C	TFS[1]
Orbital shaker platform (for suspension-based transfection protocol)	
Optional: Sf9 or Sf21 cells	
Optional: Sf-900™ II SFM or Sf-900™ III SFM	
Optional: 6-well tissue culture-treated plate	

[1] Available from Thermo Fisher Scientific.

Procedural guidelines

- We recommend isolating bacmid DNA using the PureLink™ HiPure Plasmid Prep Kits (Cat. No. K2100). To ensure sterility, you may filter your DNA preparation through a 0.22-µm filter before use.
- Allow freshly thawed cells to recover in culture for two or more passages post-thaw before transfecting.
- Gently invert the ExpiFectamine™ Sf Transfection Reagent 5-10 times before use to ensure thorough mixing.
- ExpiFectamine™ Sf/DNA complexes must be made in serum-free medium. We recommend using Opti-MEM™ I Reduced Serum Medium for the complexation reaction.
- Do **not** add antibiotics to media during transfection because it may decrease transfection efficiency.
- ExpiFectamine™ Sf Transfection Reagent can be used to transfect ExpiSf9™, Sf9, and Sf21 cells in either adherent or suspension culture format. Two different transfection protocols are included in this manual for added flexibility.
- Make sure you have a healthy cell culture at log phase growth and ≥90% viability prior to proceeding with transfection.

Approximate experimental step times

Procedure	Time
Cell preparation	0-60 minutes
Incubation of diluted lipid reagent	5 minutes
Incubation of DNA-lipid mixture	5 minutes
Cell incubation post-transfection	72-96 hours

Suspension-based transfection of ExpiSf9™, Sf9, and Sf21 cells with ExpiFectamine™ Sf Transfection Reagent using the Bac-to-Bac™ Baculovirus Expression System

Use the following procedure to transfect ExpiSf9™, Sf9, and Sf21 suspension cell cultures in a 125-mL shake flask format. All amounts are given on a per-flask basis.

1 Prepare cells and medium

a. At the time of transfection, dilute cells to 2.5×10^6 cells/mL (≥90% viability) in 25 mL of growth medium (ExpiSf™ CD Medium in a 125-mL non-baffled, vented shake flask.) Prepare the cells as follows.

Note: The following instructions are given on a per-shake flask basis.

1. Pipet 62.5×10^6 viable cells into a sterile 50-mL conical tube.
2. Centrifuge at 300 × *g* for 5 minutes.
3. Aspirate the supernatant and gently resuspend cells in 25 mL of fresh growth medium.
4. Transfer the entire cell suspension to a 125-mL shake flask.

b. Incubate cells for 0-30 minutes in a 27°C non-humidified, non-CO_2 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).

Note: It is important to proceed to Step 2 within 30 minutes of cell seeding. Incubating cells for longer than 30 minutes prior to transfection may result in decreased transfection efficiency.

2 Dilute ExpiFectamine™ Sf Transfection Reagent in Opti-MEM™ I Medium and incubate

a. Gently mix the ExpiFectamine™ Sf Transfection Reagent before use by inverting 5-10 times.

b. Dilute 30 µL ExpiFectamine™ Sf Transfection Reagent in 1 mL Opti-MEM™ I Reduced Serum Medium.

c. Gently mix by inverting 5-10 times.

d. Incubate the mixture at room temperature for 5 minutes.

3 Add Bacmid DNA directly to diluted ExpiFectamine™ Sf Transfection Reagent and incubate

a. Add 12.5 µg Bacmid DNA **directly** to the diluted ExpiFectamine™ Sf Transfection Reagent. No pre-dilution of Bacmid DNA is required.

b. Gently mix by inverting 5-10 times.

c. Incubate the mixture at room temperature for 5 minutes.

4 Add DNA-lipid complex to cells

a. Slowly transfer the mixture dropwise to the prepared 125-mL shake flask from Step 1 swirling the flask during addition to ensure uniform delivery.

b. Incubate cells in a 27°C non-humidified, non-CO_2 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) until you see signs of viral infection (typically 72-96 hours).

Note: ExpiFectamine™ Sf Transfection Reagent exhibits low cytotoxicity; therefore, no media change is required post-transfection.

Adherent-based transfection of ExpiSf9™, Sf9, and Sf21 cells with ExpiFectamine™ Sf Transfection Reagent using the Bac-to-Bac™ Baculovirus Expression System

Use the following procedure to transfect ExpiSf9™, Sf9, and Sf21 adherent cell cultures in a 6-well plate format. All amounts are given on a per-well basis.

1	Prepare cells and medium	a. At the time of transfection, seed cells at 1×10^6 cells/well (≥90% viability) in complete growth medium (ExpiSf™ CD Medium in a 6-well plate (3 mL total volume per well)). b. Allow cells to attach for 30-60 minutes in a 27°C non-humidified, non-CO_2 incubator.
2	Dilute ExpiFectamine™ Sf Transfection Reagent in Opti-MEM™ I Medium and incubate	a. Gently mix the ExpiFectamine™ Sf Transfection Reagent before use by inverting 5-10 times. b. Dilute 10 µL ExpiFectamine™ Sf Transfection Reagent in 250 µL Opti-MEM™ I Reduced Serum Medium. c. Gently mix by inverting 5-10 times. d. Incubate the mixture at room temperature for 5 minutes.
3	Add Bacmid DNA to diluted ExpiFectamine™ Sf Transfection Reagent and incubate	a. Add 1 µg Bacmid DNA **directly** to the diluted ExpiFectamine™ Sf Transfection Reagent. No pre-dilution of Bacmid DNA is required. b. Gently mix by inverting 5-10 times. c. Incubate the mixture at room temperature for 5 minutes.
4	Add DNA-lipid complex to cells	a. Slowly transfer the mixture from Step 3 dropwise to the appropriate well of the prepared 6-well plate from Step 1. b. Incubate cells in a 27°C non-humidified, non-CO_2 incubator until you see signs of viral infection (typically 72-96 hours). **Note:** ExpiFectamine™ Sf Transfection Reagent exhibits low cytotoxicity; therefore, no media change is required post-transfection.

Scaling-up or -down transfections

Refer to the table below for the volumes of ExpiFectamine™ Sf and other reagents required to transfect suspension insect cultures in different formats.

Note: The volumes given are on a per-well or per-shake flask basis.

Parameter	Culture Type			
	Suspension			
Vessel type	Deep-well plate	Vented, non-baffled shaker flask		
Vessel size	24 DWP	125 mL	250 mL	500 mL
Number of cells required	10 × 10^6 cells	62.5 × 10^6 cells	125 × 10^6 cells	250 × 10^6 cells
Culture volume to transfect	4 mL/well	25 mL	50 mL	100 mL
Shake speed[1]	250 ± 5 rpm (19-mm shaking diameter)	125 ± 5 rpm (19-mm shaking diameter) 125 ± 5 rpm (25-mm shaking diameter) 95 ± 5 rpm (50-mm shaking diameter)		
Amount of plasmid DNA	1 µg	12.5 µg	25 µg	50 µg
ExpiFectamine™ Sf Transfection Reagent volume	5 µL	30 µL	60 µL	120 µL
Opti-MEM™ I Reduced Serum Medium volume	250 µL	1 mL	2 mL	4 mL
Bacmid DNA volume[2]	2-4 µL	25-50 µL	50-100 µL	100-200 µL

[1] Optimal shake speed should be determined empirically based on the specific laboratory equipment used.
[2] Bacmid DNA volume provided assumes a stock concentration of 250-500 ng/µL

Manufacturer: Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008