GeticoFect™ IC Insect Cell Transfection Reagent Instruction Manual - Suspension Cells

Product Overview

GeticoFect™ IC transfection reagent is a proprietary cationic formulation specifically designed for DNA transfection in insect cells. The reagent has been optimized to efficiently transfect various insect cells in conjunction with the baculovirus expression system.

Composition and Storage

Product Name	Specification	Storage Condition
GeticoFect™ IC Insect Cell Transfection Reagent	0.75 mL / 1.5 mL / 15 mL	Store at 4°C; do not freeze

Required Materials

1. Provided by This Product

GeticoFect™ IC Insect Cell Transfection Reagent

2. Materials to Be Prepared by the User

Sf9/Sf21 and other insect cells
Insect cell culture medium
Opti-MEM I reduced-serum medium
Bacmid DNA
Sterile PETG flat-bottom triangular flask (125 mL)

24-well deep well plate

27°C CO_2-free incubator (non-humidified)

Orbital shaker platform (for suspension transfection)

Optional: Sf9/Sf21 cells

Other Material List

- Optional: Sf-900 SFM medium
- Optional: 6-well cell culture plate

Operation Guide

1. General Precautions

1. DNA preparation: Purify Bacmid DNA using a dedicated plasmid extraction kit and sterilize it through a 0.22 μm filter before use.
2. Cell resuscitation: Newly thawed cells should be passaged at least twice before use.
3. Reagent mixing: Gently invert the reagent bottle 5–10 times before use to ensure thorough mixing.
4. Complex preparation: Perform in serum-free medium (e.g., Opti-MEM I).
5. Antibiotic contraindication: Do not add antibiotics to the medium during transfection.
6. Cell status: Use healthy cells in the logarithmic growth phase with viability ≥90%.

2. Experimental Steps Time Estimation

Step	Time Consumption
Cell preparation	0–60 minutes
Dilution and incubation of transfection reagent	5 minutes
Formation of DNA-transfection reagent complex	5 minutes
Post-transfection culture	72–96 hours

Transfection Protocols

1. Suspension Cell Transfection Protocol (125 mL Shaker Flask)

1. Cell Preparation

- Check cell status: Observe cell morphology and density under a microscope to ensure cells are in the logarithmic growth phase with viability ≥90%. Dilute cells to \(2.5 × 10^{6}\) cells/mL in a total volume of 25 mL using a 125 mL culture flask. Determine cell viability by trypan blue staining: mix cell suspension with equal-volume 0.4% trypan blue, count under a microscope (live cells unstained, dead cells blue).
- Collect cells: Transfer cells (total \(62.5 × 10^{6}\)) to a 50 mL centrifuge tube and centrifuge at 300×g for 5 minutes, placing tubes symmetrically for balance.
- Discard supernatant: Tilt the tube and aspirate supernatant slowly, leaving 0.5–1 mL to protect the cell pellet.
- Resuspend cells: Add 25 mL fresh medium and pipette gently to form a uniform suspension, avoiding bubbles.
- Transfer cells: Move the suspension to a 125 mL ventilated flask, pre-culture on a 27°C orbital shaker at 125±5 rpm (19–25 mm amplitude) or 95±5 rpm (50 mm amplitude) for 10–30 minutes.

2. Reagent Preparation

- Prepare a sterile 1.5 mL centrifuge tube labeled "GeticoFect™ IC Reagent Dilution".
- Aspirate 30 µL of reagent with a sterile pipette, ensuring correct volume setting.
- Add the reagent to 1 mL of Opti-MEM I medium, invert 5–10 times to mix, and incubate at room temperature for 5 minutes.

3. Complex Preparation

- Add 12.5 µg of Bacmid DNA to the diluted reagent, calculate volume based on concentration, invert to mix, and incubate at room temperature for 5 minutes.

4. Transfection Operation

- Add the complex dropwise to the flask while gently rotating, then shake the flask to mix cells and complex.
- Culture on the shaker at 27°C for 72–96 hours, observing cell status (e.g., swelling, suspension as signs of viral infection).

2. Adherent Cell Transfection Protocol (6-Well Plate)

1. Cell Preparation

- Prepare a cell suspension, count cells to ensure logarithmic phase and viability ≥90%.
- Inoculate \(1 × 10^{6}\) cells per well in a 6-well plate with 3 mL medium, culture at 27°C for 30–60 minutes for adhesion.
- Check cell adhesion under a microscope before proceeding.

2. Reagent Preparation

- Prepare a sterile 1.5 mL tube, aspirate 10 µL of reagent, add to 250 µL Opti-MEM I, invert to mix, and incubate for 5 minutes.

3. Complex Preparation

- Add 1 µg of Bacmid DNA to the diluted reagent, mix by inversion, and incubate for 5 minutes.

4. Transfection Operation

- Add the complex dropwise to each well, shake the plate gently, and culture at 27°C for 72–96 hours, monitoring cell status.

Suspension Cell Transfection Scale Adjustment Guide

Parameter	**Culture Type**			
	Suspension Cells			
Container Type	24-Well Deep Plate	125 mL Flask	250 mL Flask	500 mL Flask
Cell Count Required	10 × 10^6 cells	62.5 × 10^6 cells	125 × 10^6 cells	250 × 10^6 cells
Transfection Culture Volume	4 mL/well	25 mL	50 mL	100 mL
Shaker Speed	250±5 rpm (19 mm diameter)	125±5 rpm (19 mm diameter) / 125±5 rpm (25 mm diameter) / 95±5 rpm (50 mm diameter)		
Bacmid DNA	1 µg	12.5 µg	25 µg	50 µg

Parameter	Culture Type			
GeticoFect™ IC Reagent Volume	5 µL	30 µL	60 µL	120 µL
Opti-MEM Medium Volume	250 µL	1 mL	2 mL	4 mL
Bacmid DNA Volume	2–4 µL	25–50 µL	50–100 µL	100–200 µL

Notes

- Shaker speed optimization: Determine the optimal speed through pre-experiments as equipment may vary.
- Cytotoxicity: The reagent has low cytotoxicity; medium replacement is unnecessary, but partial replacement can be considered if cell status is poor.
- Storage: Store at 4°C, avoid freezing, and equilibrate to room temperature before use.