GeticoFect™ IC-Lite Insect Cell Transfection Reagent Instruction Manual

I. Product Information

(1) Product Characteristics

Parameter	Details
Main Component	Cationic mixture
Appearance	Colorless liquid with slight opalescence
pH Value	6.8–7.2 (at 25°C)
Osmotic Pressure	280–320 mOsm/kg

(2) Storage Conditions

- Store at 4°C for up to 24 months.
- Avoid freezing, as it damages the transfection reagent structure.
- After removing from the refrigerator, equilibrate at room temperature for 30 minutes, then invert 10 times before use.

II. Experimental Preparation

(1) List of Instruments and Consumables

Item	Specification/Model	Remarks
CO_2 Incubator	371 (Thermo Fisher)	Set at 27°C, humidity 70–80%
Centrifuge	5810R (Eppendorf)	With 15 mL and 50 mL rotors
Vortex Mixer	Vortex-Genie 2 (Scientific Industries)	Adjust to low speed
Pipettes	Various specifications	Calibrated with error <1%
6-Well Plate	Corning 3516 (TC-treated)	Irradiated with UV for 30 minutes before use
Centrifuge Tubes	1.5 mL, 15 mL, 50 mL (Axygen)	Autoclaved

(2) Solution Preparation Methods

Grace's Medium (10% FBS)

- Take 85 mL Grace's basal medium.
- Add 15 mL fetal bovine serum (FBS, heat-inactivated).
- Add 1% double antibiotics (100 U/mL penicillin + 100 µg/mL streptomycin).
- Filter sterilize through a 0.22 µm membrane.
- Dispense into 50 mL centrifuge tubes and store at 4°C for no more than 2 weeks.

Plating Medium (1.5% FBS)

- Measure 1.5 mL Grace's medium with 10% FBS.
- Add 8.5 mL serum-free Grace's medium.
- Prepare fresh before use.

III. Detailed Transfection Protocol

(1) Transfecting Sf9 Cells with Bacmid

1. Cell Culture and Counting

1.1 Cell Recovery

- Remove the cryotube from liquid nitrogen and immediately place it in a 37°C water bath, shaking continuously to thaw cells within 1 minute.
- Disinfect the cryotube with 75% alcohol, then transfer the cell suspension to a 15 mL centrifuge tube.
- Slowly add 5 mL Grace's medium with 10% FBS and pipette gently to mix.
- Centrifuge at 300×g for 5 minutes and discard the supernatant.
- Resuspend cells in 10 mL fresh medium, transfer to a 25 cm^2 culture flask, and incubate at 27°C for 24 hours.

1.2 Cell Passaging

- Passage cells when density reaches 80%.
- Aspirate old medium and wash cells twice gently with PBS.
- Add 2 mL 0.05% trypsin-EDTA solution and digest at room temperature for 2 minutes.
- Terminate digestion with an equal volume of medium containing 10% FBS.
- Pipette gently to form a single-cell suspension.
- Count cells and adjust the density to 1×10^6 cells/mL.

1.3 Cell Counting Method

- Mix 10 µL cell suspension with 10 µL trypan blue solution in a 1.5 mL centrifuge tube.
- Drop the mixture into the counting chamber of a hemocytometer and let stand for 3 minutes.
- Count live cells (unstained) in four large squares under a microscope.
- Calculate cell density: Cell density (cells/mL) = (total live cells/4) × 10^4 × dilution factor.

2. Transfection Reagent Preparation

2.1 Reagent Dilution

- Remove GeticoFect™ IC-Lite from the 4°C refrigerator and equilibrate at room temperature for 30 minutes.
- Invert the reagent bottle 10 times to mix thoroughly.
- Pipette 8 µL reagent with a 10 µL pipette into a 1.5 mL centrifuge tube.
- Add 100 µL serum-free Grace's medium and pipette gently 10 times to mix.
- Vortex at low speed for 5 seconds.
- Use within 30 minutes at room temperature.

2.2 DNA Preparation

- Take 1 µg Bacmid DNA (concentration ≥0.5 µg/µL) into a 1.5 mL centrifuge tube.
- Add serum-free Grace's medium to 100 µL and pipette gently 10 times.

3. Complex Formation

3.1 Mixing

- Slowly pour the diluted DNA solution into the diluted reagent while pipetting gently.
- The total volume is ~210 µL; mix gently 3 times to avoid bubbles.

3.2 Incubation

- Let the centrifuge tube stand at room temperature for 20 minutes, flicking the tube bottom once every 5 minutes to promote complex formation.

4. Cell Transfection

4.1 Plating

- Pipette 2 mL plating medium with 1.5% FBS into each well of a 6-well plate.
- Pipette 1 mL cell suspension (8×10^5 cells) into each well and shake gently.
- Let the 6-well plate stand at room temperature for 15 minutes to allow cell attachment.

4.2 Reagent Addition

- Pipette 210 µL DNA-transfection reagent complex and add dropwise into each well from 1 cm above the cell layer.
- Add at 5 different positions per well to ensure uniform distribution.

4.3 Incubation

- Seal the 6-well plate with parafilm to prevent medium evaporation.
- Incubate at 27°C for 3 hours.
- Observe cell status hourly; replace medium immediately if it turns yellow.

5. Subsequent Culture and Detection

5.1 Medium Replacement

- After 3 hours, carefully aspirate the transfection medium without disturbing the cell layer.
- Add 2 mL Grace's medium with 10% FBS to each well.
- Antibiotics (e.g., 50 µg/mL gentamicin) can be added optionally.

5.2 Virus Detection

- Observe cell morphology daily under a microscope and record signs of viral infection (e.g., cell swelling, increased granules).
- At 72 hours post-infection, collect supernatant for virus titer determination (plaque assay or TCID50 method).
- Collect cell pellets for Western blot to confirm target protein expression.

(2) Transfecting High Five Cells with Plasmid DNA

1. Special Preparations

1.1 ER Reagent Treatment (purchased separately)

- Add 5 µL PLUS reagent to the diluted DNA solution.
- Mix gently and stand at room temperature for 5 minutes.

1.2 Medium Selection

- Express Five SFM medium is recommended to improve transfection efficiency.

2. Transfection Procedure Differences

2.1 Complex Incubation Time

- Stand at room temperature for 15 minutes; no need to extend incubation.

2.2 Medium Replacement Time

- If using SFM medium, no replacement is needed; culture directly for 24 hours.
- If using Grace's medium, replace with complete medium after 3 hours.

IV. Quality Control Standards

(1) Key Parameter Requirements

Parameter	Standard	Detection Method
DNA Purity	OD260/280 = 1.8–2.0	Spectrophotometer
Cell Viability	≥95%	Trypan blue staining
Complex Particle Size	100–200 nm	Dynamic light scattering
Transfection Efficiency	≥80% (GFP reporter gene)	Flow cytometry

V. Troubleshooting Guide

(1) Common Issues and Solutions

Problem	Possible Cause	Solution
Low transfection efficiency	Degraded DNA	Use freshly prepared DNA and verify integrity by agarose gel electrophoresis
Massive cell death	High complex concentration	Reduce reagent to 4 µL/well and extend incubation to 4 hours
Poor High Five™ cell adhesion	PLUS reagent not used	Add PLUS reagent strictly as per the manual
Low virus titer	Insufficient post-transfection culture time	Extend culture to 96 hours and lower temperature to 25°C

(2) Advanced Optimization

Gradient Experiment Design

- DNA concentration gradient: 0.5 µg, 1 µg, 2 µg, 3 µg.
- Reagent volume gradient: 4 µL, 6 µL, 8 µL, 10 µL.
- Incubation time gradient: 2 h, 3 h, 4 h, 5 h.

Electron Microscopy Observation

- Perform negative staining on transfected cells and observe complex internalization by transmission electron microscopy.

VI. Appendix

(1) Solution Formulations

Trypan Blue Stain

- Weigh 0.4 g trypan blue powder, add 100 mL normal saline, and stir to dissolve.
- Filter through filter paper to remove undissolved particles.
- Dispense into brown bottles and store at room temperature.

PBS Buffer

- Weigh 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4.

- Add 800 mL deionized water and stir to dissolve.
- Adjust pH to 7.4 with HCl.