Insect GeneJuice® Transfection Reagent

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About the Kits

Insect GeneJuice® Transfection Reagent	0.3 ml	71259-3
	1 ml	71259-4
	10 × 1 ml	71259-5

Description

Insect GeneJuice Transfection Reagent is a proprietary liposome formulation optimized for maximal transfection efficiency of *Spodoptera frugiperda*-derived insect cells (e.g. Sf9 or Sf21). The reagent features extremely low cytotoxicity and can be used for both transient and stable transfections in serum-containing or serum-free media. Insect GeneJuice Transfection Reagent is ideal for large-scale protein expression in Sf9 suspension cultures when using Novagen's pIEx™ and pBiEx™ Vectors (1). One milliliter Insect GeneJuice Transfection Reagent is sufficient for 10 or 100 transfections in 10-ml suspension cultures or 35-mm plates, respectively.

Components

- 0.3 or 1 or 10 × 1 ml Insect GeneJuice Transfection Reagent (2 mg/ml suspension in 150 mM NaCl, 10mM MES, pH 6.2)

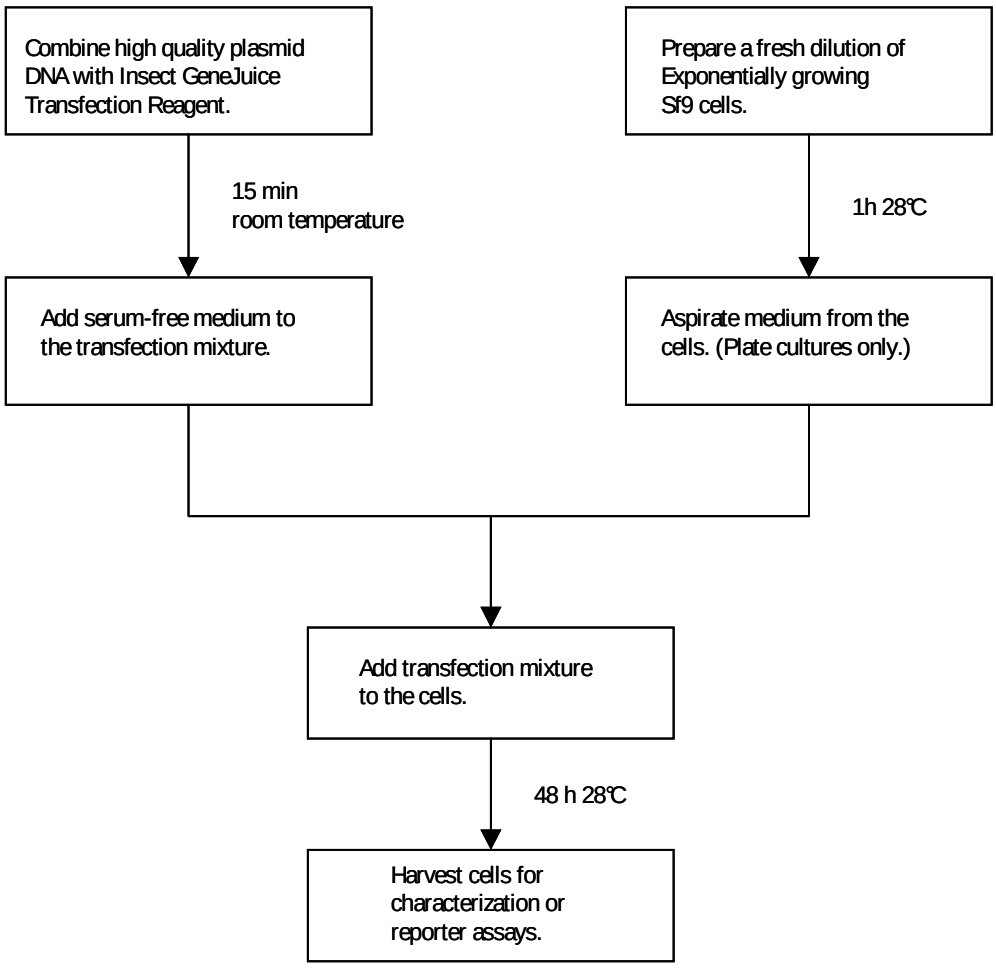
Storage

Store Insect GeneJuice Transfection Reagent at 4°C.

General Considerations

- Use only high quality DNA. If necessary, include an endotoxin removal step. The prepared DNA should be re-suspended in TE buffer (10mM Tris-HCl, 1 mM EDTA, pH 8.0) or TlowE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) at a concentration of 1 µg/µl.
- Passage cells regularly (e.g., every 2–3 days) and avoid confluent growth. For transfection, use only rapidly proliferating cells. Conditions for cell growth and density should be consistent for optimum reproducibility.
- Sf9 Insect Cells (Cat. No. 71104-3) plus BacVector® Insect Cell Medium (Cat. No. 70590-3) are recommended for transfection and for baculovirus plaque assays. While TriEx™ Sf9 Cells (Cat. No. 71023-3) can be used for transient or stable transfections, they may give lower transfection efficiencies than Sf9 Insect Cells.
- Insect GeneJuice® Transfection Reagent is compatible with both serum-containing and serum-free media. Note that serum must not be present during formation of the DNA/Insect GeneJuice complex.
- The ratio of Insect GeneJuice Transfection Reagent to DNA is a crucial factor for transfection optimization. We recommend 5 µl Insect GeneJuice Transfection Reagent per 1 µg DNA as a starting point. For optimization, vary between 4–8 µl Insect GeneJuice per 1 µg DNA.

Experimental outline



Plasmid Transfection

The following procedures describe methods for introduction of plasmid DNA into Sf9 cell cultures in a 24-well and 10-ml suspension culture formats. Alternative formats require adjustment of the amount of Insect GeneJuice® Transfection Reagent and cell seeding densities.

The following table lists the amount of cells and reagents for 3 different tissue culture formats.

Transfection of Plasmid DNA	**Tissue Culture Format**		
	Plate format (well/plate)		**Flask format**
	24-well	**6-well**	**10 ml (125 ml flask)**
Number of cells	2×10^5	1×10^6	1×10^7
Culture volume prior to transfection	0.5 ml	2.5 ml	8 ml
Amount of plasmid DNA	0.4 µg	2 µg	20 µg
Volume of Insect GeneJuice® Transfection Reagent	2 µl	10 µl	100 µl
Volume of serum-free medium added to both the plasmid DNA **and** the Insect GeneJuice Transfection Reagent for dilution	20 µl	100 µl	1 ml
Volume of serum-free medium added to Insect GeneJuice transfection mixture after 15 min incubation	160 µl	0.8 ml	N/A

24-well plate culture

Cell preparation

1. Make 15 ml of a fresh dilution of Sf9 cells from an exponentially growing shake culture. Dilute the cells in prewarmed (28°C) serum-free medium to 4×10^5 cells/ml.
2. One hour prior to transfection, add 0.5 ml (2×10^5 cells) to each well of a 24-well plate.

Transfection

1. For each well to be transfected, dilute 0.4 µg DNA with 20 µl serum free medium. Also, dilute 2 µl of Insect GeneJuice Transfection Reagent with 20 µl serum free medium.
2. Slowly add the diluted DNA *dropwise* to the diluted Insect GeneJuice Transfection Reagent. Mix immediately by gentle vortexing to avoid precipitation.
3. Incubate the Insect GeneJuice/DNA mixture at room temperature 15 min.
4. After the 15 min incubation, add 160 µl serum-free medium to the Insect GeneJuice/ DNA transfection mixture.
5. Aspirate medium from the cells and add the transfection mixture to the cells.
6. Add the cover to the plate and carefully transfer to a flat-bottomed covered storage container containing a damp paper towel for moisture. Incubate the cells for 48 h at 28°C.
   Optional: Remove transfection mixture after 4 hours and replace with complete growth medium.
7. Harvest cells for characterization or reporter assays.

Note: *Novagen's Insect PopCulture® Reagent (Cat. No. 71187) is ideal for the extraction of proteins from insect cells directly in their culture medium without mechanical disruption or centrifugation. Cytobuster™ Protein Extraction Reagent (Cat. No.71009) can be used for the extraction of soluble proteins from insect cell monolayer cultures or cell pellets. Reportasol™ Extraction Buffer (Cat. No. 70909) can also be used for extraction of protein from insect cell monolayer culture or cell pellets and is optimized for maximal activity of reporter enzymes.*

10 ml suspension culture

1. Seed 1×10^7 Sf9 cells in 8 ml serum-free medium in 125 ml Erlenmeyer flask.
2. In a sterile tube, dilute 20 µg plasmid DNA with 1 ml of serum-free medium. Also, dilute 100 µl Insect GeneJuice Transfection Reagent with 1 ml of serum-free medium.
3. Add the DNA *dropwise* to the Insect GeneJuice Transfection Reagent and mix immediately by gentle vortexing to avoid precipitation.
4. Incubate at room temperature 15 min.
5. Add the transfection mixture to the cells.
6. Incubate the cells for 48 h at 28°C, shaking at 150 rpm.
7. Harvest cells for characterization or reporter assays.

Troubleshooting

Symptom	Possible cause	Solution
Low transfection efficiency	Serum present during formation of Insect GeneJuice/DNA complex	Use only serum-free medium during formation of complex. If cells were grown in presence of serum, wash the cells once before adding transfection mix.
	Cell density suboptimal at time of transfection	Use cell densities outlined in the General Considerations Section.
	Poor quality DNA	Prepare fresh plasmid DNA by including a step to remove endotoxins.Alternatively, prepare supercoiled plasmid DNA by using a CsCl/EtBr protocol.
	Inhibitor present during transfection	In addition to endotoxin, transfection can be inhibited by the presence of polyanions such as heparin or dextran sulfate. Be sure that the DNA and the transfection medium are free of polyanions.

References

1. Loomis, K., Novy, R. and Yaeger, K. (2003) *in**Nova**tions* **16**, 7–10.