GeneJuice® Transfection Reagent

Description

GeneJuice® Transfection Reagent	0.3 ml	70967-5
	1 ml	70967-3
	5 x 1 ml	70967-6
	10 x 1 ml	70967-4

GeneJuice® Transfection Reagent is a proprietary formulation optimized for maximal transfection efficiency, ease of use, and minimal cytotoxicity. This transfection reagent is superior to a wide variety of other techniques including calcium phosphate coprecipitation, electroporation, microinjection, biolistic particle delivery, and complex formation with DEAE-dextran.

Whereas many available transfection reagents are based on cationic lipid formulation, GeneJuice Transfection Reagent is composed of a nontoxic cellular protein and a small amount of a novel polyamine. GeneJuice Transfection Reagent enables highly efficient DNA transfer in both stable and transient transfection of eukaryotic cells. The unique composition is compatible with both serum-containing and serum-free media, making media changes unnecessary. GeneJuice Transfection Reagent is ideal for high-throughput transfections in a multi-well plate format.

The 1-ml size provides enough reagent to perform up to 500 transfections in standard 35-mm plates. An introductory 0.3-ml size is also available. GeneJuice Transfection Reagent is supplied as a ready-to-use sterile solution.

Additional information is available at www.novagen.com/transfection. Select "Citations for Transfection Reagent Usage" for an up-to-date list of cell lines successfully transfected with GeneJuice, along with associated references.

Components

- 0.3 or 1 or 5 or 10 × 1 ml — GeneJuice Transfection Reagent (1.33 mg/ml suspension in 80–90% ethanol)

Storage

Store GeneJuice tightly capped at 4°C.

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General considerations

- Use only high-quality DNA. GeneJuice® Transfection Reagent has been optimized for use with DNA purified using Novagen Mobius™ and UltraMobius™ Plasmid Kits (Cat. Nos. 70853-3 and 70906-3).
- Passage cells regularly (e.g., every 2–3 days). Avoid confluent growth. Use only rapidly proliferating cells for transfection. For optimum reproducibility, conditions for cell growth and density should be consistent.
- GeneJuice Transfection Reagent is compatible with both serum-containing and serum-free media.

Note: Serum must not be present during formation of the GeneJuice reagent/DNA complex.

Transfection of Plasmid DNA	Tissue Culture Format					
	Plate format (wells/plate)			Dish format (mm)		
	24	12	6	35	60	100
Number of adherent cells ($\times 10^5$)	0.2–0.8	0.5–1.0	1–3	1–3	5–10	15–25
Number of suspension cells ($\times 10^5$)	2–4	4–8	10–20	10–20	28–56	80–160
Volume of complete growth medium in well or dish (ml)	0.5	1	3	3	5	15
Volume of serum-free medium in transfection mixture (µl)	20	50	100	100	300	800
Volume of GeneJuice® Transfection Reagent (µl)	0.75	1.5	3	3	15	18
Amount of plasmid DNA (µg)	0.25	0.5	1	1	5	6

Optimization

Although GeneJuice Transfection Reagent demands much less optimization than alternative transfection reagents, determining ideal conditions for highest transfection efficiency with GeneJuice reagent is still important. Optimization is suggested for every new combination of cell line and plasmid. The most important parameters are cell density and ratio of transfection reagent to DNA.

Cell density

Optimum cell density for transfection is normally 50–80% confluency for adherent cells, and $1.0–2.0 \times 10^6$ cells/ml for suspension cells. Cell density at the time of transfection will depend on initial plating density, cell growth rate (doubling time), and length of time between plating and transfection. To ensure reproducibility, optimum cell density should be determined for every new cell line and applied in all experiments with that line. Cell density will also influence optimum quantities of DNA and GeneJuice Transfection Reagent used per well.

Ratio of GeneJuice Transfection Reagent to DNA

The ratio of GeneJuice Transfection Reagent to DNA is a crucial factor for transfection optimization. We recommend 3 µl GeneJuice reagent per 1 µg DNA as a starting point, which should give satisfactory results for the majority of cell lines. During optimization, use a range of 2–6 µl GeneJuice reagent per 1 µg DNA. The amount of DNA can also be varied from 1–3 µg DNA for transfection per 35-mm dish or per well of a multiwell plate.

Transfection Procedure (35 mm plates)

The following procedures facilitate efficient introduction of DNA into adherent and suspended eukaryotic cells. These methods are suitable for a range of cell types, but may require optimization for individual cell lines, growth conditions, and other application-specific variables. Refer to *Optimization* on p 2. The DNA to be transfected should be at a concentration of 0.5–1 µg/µl. If the DNA concentration is lower, decrease the volume of serum-free medium in the transfection mix to compensate for the larger volume of DNA.

Transfection of adherent cells

1. The day before transfection, plate 1–3 × 10^5 cells in complete growth medium per 35-mm dish. Incubate at 37°C (5% CO_2) overnight. Cells should be 50–80% confluent before transfection.
2. For each 35-mm dish to be transfected, place 100 µl serum-free medium (for example, RPMI 1640 or Opti-MEM®) into a sterile tube. Add 3 µl GeneJuice® Transfection Reagent drop-wise directly to the serum-free medium. Mix thoroughly by vortexing. Volumes can be scaled up for transfection of multiple dishes with the same DNA.
3. Mix thoroughly by vortexing.

Note: For most cell lines, the optimal ratio of GeneJuice reagent to DNA is 3 µl reagent to 1 µg DNA. However, the ratio can be varied from 2–6 µl per µg DNA during optimization.

4. Incubate at room temperature for 5 min.
5. For each 35-mm dish to be transfected, add 1 µg DNA to GeneJuice reagent/serum-free medium mixture. Mix by gentle pipetting.
6. Incubate GeneJuice reagent/DNA mixture at room temperature for 5–15 min.
7. Add entire volume of GeneJuice reagent/DNA mixture drop-wise to cells in complete growth medium. Distribute drops over entire surface of dish. Gently rock dish to ensure even distribution. Do not swirl plate, as doing so will concentrate transfection mixture in center of plate.
   Optional: Remove transfection mixture after 2–8 h incubation and replace with complete growth medium.
8. Incubate cells for 24–72 h at 37°C (5% CO_2).
9. Harvest cells for analysis.

Note: For stable cell line selection, subculture the cells (dilute at least 1:5) in complete growth medium plus selective agent. Continue incubation for 1–2 weeks, allowing for growth and selection of desired cells.

Transfection of suspension cells

1. The day before transfection, dilute cells to a density of 0.5–2.5 × 10^5 cells per ml, so they will be in log phase growth the following day. Incubate cells at 37°C (5% CO_2) overnight.
2. Plate 3 ml cells at a density of 1–2 × 10^6 cells/ml in a 35-mm dish.
3. For each 35-mm plate to be transfected, place 100 µl serum-free medium (e.g., RPMI 1640 or Opti-MEM) into a sterile tube. Add 3 µl GeneJuice Transfection Reagent drop-wise directly to serum-free medium. Mix thoroughly by vortexing. Volumes can be scaled up for transfection of multiple dishes with the same DNA.
4. Incubate at room temperature for 5 min.
5. For each 35-mm dish to be transfected, add 1 µg DNA to GeneJuice reagent/serum-free medium mixture. Mix by gentle pipetting.
6. Incubate GeneJuice reagent/DNA mixture at room temperature for 5–15 min.
7. Add entire volume of GeneJuice reagent/DNA mixture drop-wise to cells in complete growth medium. Distribute drops over entire surface of the dish. Gently rock dish to ensure even distribution. Do not swirl plate, as doing so will concentrate transfection mixture and cells in center of plate.
8. Incubate cells for 24–72 h at 37°C (5% CO_2).
9. Harvest cells for analysis.

Transfection in the absence of serum

For certain cell types, absence of serum during the transfection incubation may be advantageous.

Note: To perform transfections in absence of serum, wash cells with serum-free medium before transfecting.

1. Prepare GeneJuice® reagent/DNA transfection mix, as described in preceding sections.
2. Add transfection mix directly to cells in serum-free medium.
3. Incubate cells with transfection mixture for 2–8 h.
4. Replace transfection mixture with complete growth medium.
5. Incubate an additional 16–70 h before harvesting for analysis.

For a list of citations including culture conditions, volume of GeneJuice Transfection Reagent, and amount of DNA used, refer to www.novagen.com/transfection.

Troubleshooting

Symptom	Possible cause	Solution
Precipitate forms after adding GeneJuice reagent	GeneJuice reagent/DNA concentration too high	Increase volume of serum-free medium in transfection mixture to 200–1000 µl.
Low transfection efficiency	Serum present during formation of GeneJuice reagent/DNA complex	Use only serum-free medium during formation of complex. If cells were grown in presence of serum, wash cells once before adding serum-free medium and transfection mix.
	Cell density is suboptimal at time of transfection	Optimal cell density should be determined for each cell type. Try higher and lower cell densities.
	Ratio of GeneJuice reagent to DNA is suboptimal	Titrate GeneJuice reagent from 2 µl/µg to 6 µl/µg while holding DNA level constant at 1 µg per 35-mm dish. Alternatively or in addition, keep ratio constant but use greater volumes of GeneJuice reagent/DNA complex per 35-mm dish, from 1 to 3 µg DNA.
	Poor quality DNA	Prepare fresh plasmid DNA. Include an endotoxin removal step, such as with UltraMobius™ 1000 Plasmid Kit. Alternatively, prepare supercoiled plasmid DNA using a CsCl/EtBr protocol.
	Inhibitor present during transfection	Ensure DNA and transfection medium are free of polyanions, such as heparin or dextran sulfate.
High cell toxicity	Incomplete mixing of GeneJuice reagent/DNA complexes with cells	Distribute transfection mixture evenly to all cells on plate. Transfection mix should be added drop-wise across surface of medium. Plate should be rocked back and forth to mix. Do not swirl or rotate dish, as doing so may concentrate GeneJuice reagent/DNA complexes in center of dish.
	Excessive amount of GeneJuice reagent/DNA in transfection	Reduce amount of GeneJuice reagent/DNA complex used per dish. Remove transfection mixture after 2–8 h incubation, and replace with complete growth medium.
	Cell density too low at time of transfection	Plate more cells to achieve 50-80% confluency at time of transfection.