Novagen®

GeneJuice® Transfection Reagent

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About the Product

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

Description

GeneJuice Transfection Reagent is a proprietary formulation optimized for maximal transfection efficiency, ease of use, and minimal cytotoxicity.

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.

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 Transfection Reagent tightly capped at 4 C.

General Considerations

- Use only high-quality DNA. If necessary, include an endotoxin removal step.
- 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 Transfection Reagent/DNA complex.

Optimization

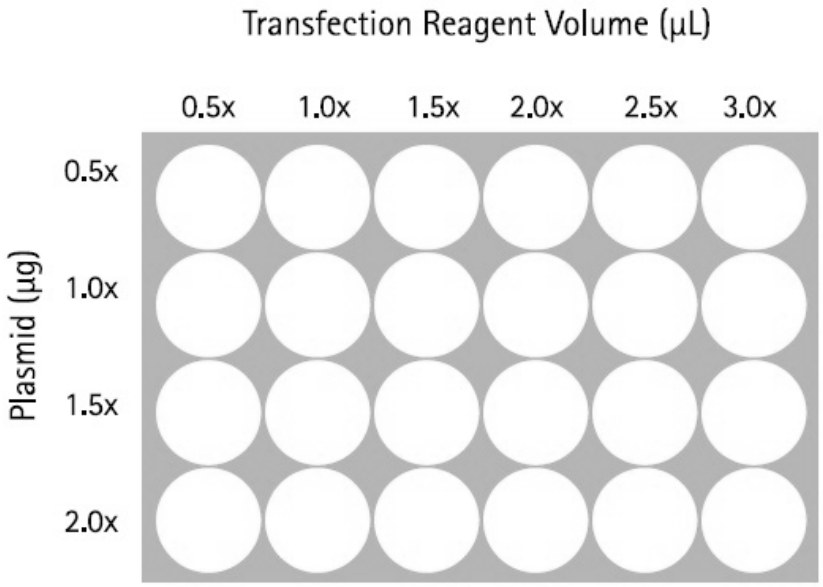
Although GeneJuice Transfection Reagent demands much less optimization than alternative transfection reagents, determining ideal conditions for highest transfection efficiency with GeneJuice Transfection Reagent is still important. Optimization is suggested for every new combination of cell line and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

Cell density

Optimum cell density for transfection for most cell types is 50 80% confluency for adherent cells, and 1.0–2.0 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. If this condition does not yield the desired results, an optimization experiment can be performed. In a 24 well plate (example below), plate the same amount of cells in each well.Set up a gradient across the plate and add the appropriate volume of transfection reagent (0.5x, 1x, 1.5x, 2x, 2.5x and 3x).Set up a gradient down the plate and add the appropriate amount of plasmid (0.5x, 1x, 1.5x and 2x). With a reporter gene in the plasmid, the optimal condition can be easily determined.



Transfection Procedure

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 page 3.

Transfection of adherent cells

	Tissue Culture Format							
	Plate format (wells/plate)					**Dish format (mm)**		
	96	48	24	12	6	35	60	100
Number of adherent cells (1 X 10^5)	0.05-0.2	0.1-0.4	0.2 - 0.8	0.5 - 1.0	1 - 3	1 - 3	5 - 10	15 - 25
Volume of complete growth medium in well or dish (ml)	0.13	0.25	0.5	1	3	3	5	10
Volume of serum-free medium in transfection mixture (µl)	6.3	12.5	25	50	100	100	250	500
Volume of GeneJuice® Transfection Reagent (µl)	0.18-0.36	0.36-0.75	0.75-1.5	1.5-3	3-6	3-6	6 - 12	15-30
Amount of plasmid DNA (µg)	0.06-0.12	0.12- 0.25	0.25-0.5	0.5-1	1-2	1-2	3-6	5 - 10

*Note: Count and plate the recommended number of cells 12-24 hours before transfection.

The following procedure describes how to perform plasmid DNA transfection for adherent cells in a 6 well plate.

1. The day before transfection, plate 1–3 10^5 cells in complete growth medium per well of a 6 well plate. Incubate at 37 C (5% CO_2) overnight. Cells should be 50–80% confluent before transfection.

   Note: For most cell lines, the optimal ratio of GeneJuice Transfection 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.

2. For each well to be transfected, place 100 µl serum-free medium into a sterile tube. Add 3 µl GeneJuice Transfection Reagent drop-wise directly to the serum-free medium. Volumes can be scaled up for transfection of multiple wells with the same DNA.
3. Mix thoroughly by vortexing.
4. Incubate at room temperature for 5 min.
5. For each well to be transfected, add 1 µg DNA to GeneJuice Transfection Reagent/serum-free medium mixture. Mix by gentle pipetting. Do NOT vortex.
6. Incubate GeneJuice Transfection Reagent/DNA mixture at room temperature for 5–15 min.
7. Add entire volume of GeneJuice Transfection 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

	Tissue Culture Format							
	Plate format (wells/plate)					Dish format (mm)		
	96	48	24	12	6	35	60	100
Number of suspension cells (1 X 10^5)	0.5-1	1-2	2 - 4	4 - 8	10 - 20	10 - 20	28 - 56	80 - 160
Volume of complete growth medium in well or dish (ml)	0.13	0.25	0.5	1	3	3	5	10
Volume of serum-free medium in transfection mixture (µl)	6.3	12.5	25	50	100	100	250	500
Volume of GeneJuice® Transfection Reagent (µl)	0.18-0.36	0.36-0.75	0.75-1.5	1.5-3	3-6	3-6	6 - 12	15-30
Amount of plasmid DNA (µg)	0.06-0.12	0.12- 0.25	0.25-0.5	0.5-1	1-2	1-2	3-6	5 - 10

*Note: Count and plate the recommended number of cells 12-24 hours before transfection.

The following procedure describes how to perform plasmid DNA transfection for suspension cells in a 6 well plate.

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 each well of a 6 well plate.

   Note: For most cell lines, the optimal ratio of GeneJuice Transfection 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.
3. For each well to be transfected, place 100 µl serum-free medium into a sterile tube. Add 3 µl GeneJuice Transfection Reagent drop-wise directly to serum-free medium. Volumes can be scaled up for transfection of multiple wells with the same DNA.
4. Mix thoroughly by vortexing.
5. Incubate at room temperature for 5 min.
6. For each well to be transfected, add 1 µg DNA to GeneJuice Tranfection Reagent/serum-free medium mixture. Mix by gentle pipetting. Do NOT vortex.
7. Incubate GeneJuice Transfection Reagent/DNA mixture at room temperature for 5–15 min.
8. Add entire volume of GeneJuice Transfection 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.
9. Incubate cells for 24–72 h at 37 C (5% CO_2).
10. 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 Transfection 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.

Troubleshooting

Symptom	Possible cause	Solution
Precipitate forms after adding GeneJuice® Transfection 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	Follow optimization protocol on page 3 to identify the optimal condition for your particular cell type and plasmid.
	Poor quality DNA	Prepare fresh plasmid DNA by including a step to remove endotoxins. Alternatively, prepare supercoiled plasmid DNA using a CsCl/EtBr protocol.
	Inhibitor present during transfection	In addition to endotoxins, 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.
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. Follow optimization protocol on page 3 to identify the optimal condition for your particular cell type and plasmid. 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.