NanoJuice™ Transfection Reagents and Kits

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USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Europe

France	Germany	Ireland	United Kingdom	All other European Countries
Freephone	Freecall	Toll Free	Freephone	+44 115 943 0840
0800 126 461	0800 100 3496	1800 409 445	0800 622 935	

techservice@merckbio.eu

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

www.novagen.com

About the Kit

NanoJuice™ Transfection Kit		71902-3 71902-4
NanoJuice Core Transfection Reagent	1 ml	71900-3
NanoJuice Transfection Booster	1 ml	71901-3

Description

The NanoJuice™ Transfection Kit combines the leading edge nanotechnology of Priostar®dendrimers with a polycationic liposomal formulation for mammalian cell transfection. This unique combination is designed for maximal transfection efficiency and minimal cytotoxicity with mammalian cells, especially primary cells, Saos-2, Caco-2, HUVEC, Jurkat, RAW264.7, HeLa, and other difficult-to-transfect mammalian cells.

The transfection conditions for mammalian cells vary greatly depending on the cell line. Primary cells and other difficult-to-transfect cell lines are particularly challenging in this regard, necessitating extensive optimization. Whereas current transfection methods rely on single reagents limiting the scope of optimization, the NanoJuice Transfection Kit consists of two components: NanoJuice Core Transfection Reagent and NanoJuice Transfection Booster. These two components can be combined in different ratios with plasmid DNA to create a reagent customized for transfecting specific cell types. Higher transfection efficiencies are attained with the NanoJuice Transfection Kit when compared to conventional transfection reagents based on either liposomal lipids or dendrimers alone. Other important benefits of this kit include:

- Low cytotoxicity
- Elimination of the need for media changes
- Reagents are derived from non-animal sources
- Compatibility with both serum-containing and serum-free media
- Flexibility in reaction conditions allows optimization for specific cell types
- Suitability for both stable and transient transfections

The NanoJuice Transfection Kits provide sufficient reagents for 240 (71902-3) or 2400 (71902-4) transfections in a 24-well format. NanoJuice Core Transfection Reagent and NanoJuice Transfection Booster are available separately as ready-to-use sterile solutions in 1-ml sizes.

Components

NanoJuice™ Transfection Kit

100 µl or 1 x 1 ml	NanoJuice Core Transfection Reagent
200 µl or 2 x 1 ml	NanoJuice Transfection Booster

Storage

Store tightly capped at 4°C.

General Considerations

- Use only high-quality, endotoxin-free DNA. DNA 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 protocol to compensate for the larger volume of DNA.
- Passage cells regularly (e.g., every 2–3 days). Avoid confluent growth. Use only rapidly proliferating cells for transfection. To ensure reproducibility, keep cell growth conditions and density consistent.
- NanoJuice™ Transfection Reagents are compatible with both serum-containing and serum-free media.
- Avoid the use of antibiotics during transfection. Increased cell permeability during transfection causes high antibiotic influx, thereby harming cell growth.

Note: Do not include serum and antibiotics during the formation of the transfection mixture.

Initial NanoJuice™ Reagent Screening Experiment

Since transfection conditions vary greatly between cell lines, we recommend performing an initial reagent screening experiment in order to attain the best results for a given cell line. Determine the optimum ratio of NanoJuice Core Transfection Reagent and NanoJuice Transfection Booster to DNA for your target cell line by combining various amounts of each reagent with a constant amount of plasmid DNA. The typical range for NanoJuice Reagents per µg DNA is 1–2 µl NanoJuice Core Transfection Reagent and 1–4 µl NanoJuice Transfection Booster. If the best transfection results are obtained with reagent volumes at the top or bottom of the range for either component in the initial experiment, perform a subsequent experiment to determine the optimal amount of each component. A sample protocol for performing the initial optimization experiment in a single 24-well plate is given below.

Example reagent screening experiment for adherent cells (24-well plate format)

For adherent cells, use the following protocol to screen 8 different ratios of NanoJuice Core Transfection Reagent and NanoJuice Transfection Booster (in triplicate) with a fixed amount of DNA per well in a 24-well plate. Please see Table 1 on p 4.

1. The day before transfection, plate 2–8 × 10^4 cells in 0.5 ml complete growth medium per well in all wells of a 24-well plate. Incubate at 37°C (5% CO_2) overnight. Cells should be 50–80% confluent at the time of transfection.
2. As indicated in Table 1 on p 4, prepare a master mix for each set of triplicate wells by placing 64 µl serum-free medium (e.g., RPMI 1640 or Opti-MEM®) in each of 8 labeled, sterile tubes. Please see step 5 regarding volume adjustment of serum-free medium.
3. As indicated in Table 1 on p 4, add the specified amount of NanoJuice Core Transfection Reagent followed by NanoJuice Transfection Booster.
4. Incubate NanoJuice reagents and medium mixture at room temperature for 5 min.
5. Add 0.8 µg DNA to each master mix tube. Mix by gentle pipetting. Initial DNA concentration should be between 0.5–1 µg/µl. If the DNA concentration is lower, decrease the volume of serum-free medium in the transfection mixture (see step 2) to compensate for the larger volume of DNA.
6. Incubate transfection mixture at room temperature for 15 min.
7. From each tube, add 20 µl transfection mixture to each of 3 wells in the prepared 24-well plate in a
   drop-wise fashion. The final amount of DNA is approximately 0.25 µg DNA per well.
8. Distribute the transfection mixture evenly by rocking the plate. Do not swirl plate, as doing so will concentrate transfection mixture in well centers.
9. Incubate cells for 24–72 h at 37°C (5% CO_2).
10. Harvest cells for characterization or reporter assays.

Example reagent screening experiment for suspension cells (24-well plate format)

For suspension cells, use the following protocol to screen 8 different ratios of NanoJuice Core Transfection Reagent and NanoJuice Transfection Booster (in triplicate) with a fixed amount of DNA per well in a 24-well plate. Please see Table 1 on p 4.

1. The day before transfection, seed the suspension cell culture at a density of 0.5–2.5 × 10^5 cells per ml in complete growth medium, so they will be in log phase growth the following day. Incubate cells at 37°C (5% CO_2) overnight.
2. On the day of transfection, plate cells at a density of 2–5 × 10^5 cells per well in 0.5 ml complete growth medium in all wells of a 24-well plate.
3. Prepare 8 tubes of transfection mixture master mixes, as described in Steps 2–5 of *Example reagent screening experiment for adherent cells* on p 3.
4. From each tube, add 20 µl transfection mixture each to 3 wells in the prepared 24-well plate. The final amount of DNA is approximately 0.25 µg DNA per well. Mix transfection mixture with cells by pipetting.
5. Incubate cells for 24–72 h at 37°C (5% CO_2).
6. Harvest cells for characterization or reporter assays.

Table 1. Combinations of NanoJuice™ Core Transfection Reagent, NanoJuice Transfection Booster and DNA to use for preparing master mix for screening experiments. Add components in the order given (i.e., medium, NanoJuice Core Transfection Reagent, NanoJuice Transfection Booster, DNA). Each tube includes sufficient volume to test transfections in triplicate in a 24 well format.

Tube number	1	2	3	4	5	6	7	8
(1) Volume serum-free medium (µl)	64	64	64	64	64	64	64	64
(2) Volume NanoJuice Core Transfection Reagent (µl)	0.8	1.6	0.8	1.6	0.8	1.6	0.8	1.6
(3) Volume NanoJuice Transfection Booster (µl)	0.8	0.8	1.6	1.6	2.4	2.4	3.2	3.2
(4) Amount DNA (µg)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Final NanoJuice Core Transfection Reagent: DNA ratio	1	2	1	2	1	2	1	2
Final NanoJuice Transfection Booster: DNA ratio (µl/µg)	1	1	2	2	3	3	4	4

Note: If pipetting small volumes (<1 µl) is undesirable, dilute each NanoJuice component and/or the DNA in serum-free medium prior to adding to transfection mix. Decrease the volume of serum-free medium in the transfection mix accordingly.

Cell Transfection Protocol

Once the suitable ratios of NanoJuice reagents to DNA have been determined, use the following protocols for transfecting cells in a 24-well format. For culture vessels of various sizes, refer to Table 2 on p 5 for appropriate volumes of each reagent. This protocol is suitable for a range of cell types but may require optimization for particular cell lines, growth conditions, and other application-specific variables. Refer to *Optimization Experiments* on p 5 for more details.

Transfecting adherent cells (24-well plate format)

1. The day before transfection, plate 2–8 × 10^4 cells in 0.5 ml complete growth medium per well of a
24-well plate. Incubate at 37°C (5% CO_2) overnight. Cells should be 50–80% confluent at the time of transfection.
2. For each well to be transfected, place 20 µl serum-free medium (e.g., RPMI 1640 or Opti-MEM®) into a sterile tube. Please see step 5 regarding volume adjustment of serum-free medium.
3. Add NanoJuice Core Transfection Reagent and NanoJuice Transfection Booster (as determined in the initial reagent screening step on p 3) and mix thoroughly. Volumes can be scaled up to prepare master mixes for transfection of multiple wells with the same DNA.
4. Incubate the NanoJuice reagents and medium mixture at room temperature for 5 min.
5. Add 0.25 µg DNA to the NanoJuice reagents and medium mixture. Mix by gentle pipetting. DNA 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 mixture (see step 2) to compensate for the larger volume of DNA.
6. Incubate transfection mixture at room temperature for 15 min.
7. Add entire volume of transfection mixture to cells drop-wise. Distribute the transfection mixture evenly by rocking the plate. Do not swirl plate, as doing so will concentrate

transfection mixture in well centers. **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 characterization or reporter assays.

Transfection of suspension cells (24-well plate format)

1. The day before transfection, seed the suspension cell culture at a density of 0.5–2.5 × 10^5 cells per ml in complete growth medium, so they will be in log phase growth the following day. Incubate cells at 37°C (5% CO_2) overnight.
2. On the day of transfection, plate cells at a density of 2–5 × 10^5 cells per well in 0.5 ml complete growth medium in a 24-well plate.
3. Prepare transfection mixture, as described in Steps 2–5 of *Transfecting adherent cells* on p 4.
4. Add entire volume of transfection mixture to cells. Mix transfection mixture with cells by pipetting.
5. Incubate cells for 24–72 h at 37°C (5% CO_2).
6. Harvest cells for characterization or reporter assays.

Transfection in absence of serum (24-well plate format)

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

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

1. Prepare the transfection mixture, as described in Steps 2–5 of *Transfecting adherent cells* on p 4.
2. Add entire volume of transfection mixture 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.

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.

Table 2. Preparation of Transfection Mixtures for Various Culture Formats.

Transfection of Plasmid DNA	Tissue Culture Format					
	Plate Format (wells/plate)			Dish Format (mm)		
	24	12	6	35	60	100
Number of adherent cells (× 10^5)	0.2–0.8	0.5–1.0	1–3	1–3	5–10	15–25
Number of suspension cells (× 10^5)	2–5	4–8	10–20	10–20	28–56	80–160
Volume complete growth medium (ml)	0.5	1	3	3	5	15
Volume serum-free medium (µl)	20	50	100	100	300	800
Volume NanoJuice™ Core Transfection	0.25–0.5	0.5–1	1.25–2.5	1.25–2.5	5–10	6–12
Volume NanoJuice–Transfection Booster (µl)	0.25–1	0.5–2	1.25–4	1.25–4	5–20	6–24
Amount of plasmid DNA (µg)	0.25	0.5	1.25	1.25	5	6

Note: If the culture format is changed to a different scale, the ratio of NanoJuice™ reagents to DNA does not need to be redetermined.

Optimization Experiments

The following parameters may be tested to improve transfection efficiencies for specific cell types:

- Test NanoJuice Core Transfection Reagent and NanoJuice Transfection Booster volumes outside the range of initial reagent screening experiment. If the initial screening experiment shows the best transfection results with reagent volumes at the top or bottom of the range for either component, try using amounts that are lower or higher, respectively. For example, if optimal volumes in the initial experiment are 1 µl of Core Reagent and 4 µl of Transfection

Booster per µg DNA, extend the range to 0.5–0.75 µl of Core Reagent and 4.5–5 µl of Transfection Booster per µg DNA.

- The order in which the NanoJuice reagents are added to DNA can influence transfection complex formation. Try changing the order in which reagents are added to the serum-free media, e.g.,
  1) NanoJuice Core Transfection Reagent, 2) NanoJuice Transfection Booster, 3) DNA (order used in initial reagent screening experiment), or
  1) DNA, 2) NanoJuice Core Transfection Reagent, 3) NanoJuice Transfection Booster, or
  1) DNA, 2) NanoJuice Transfection Booster, 3) NanoJuice Core Transfection Reagent.
- Increase the amount of DNA. Transfecting 0.5–1 µg DNA per well of a 24-well plate may enhance transfection for certain cell types. When increasing the amount of DNA, increase the amount of each NanoJuice™ Transfection Reagent proportionately.
- Increase cell-seeding density. For most cell types, 50–80% confluency at transfection is optimum, but greater starting density may give better results for some cell types.
- Perform transfection in absence of serum for 2–8 h. See detailed protocol on p 5.
- Remove medium containing transfection mixture from cells and replace with fresh, serum-containing medium 2–8 h post-transfection.

Troubleshooting Guide

Symptom	Possible cause	Solution
Low transfection efficiency	Ratio of NanoJuice reagents to DNA is suboptimal	Optimize the ratios of NanoJuice reagents to DNA according to the Initial NanoJuice Reagent Screening Experiment on p 3. If the optimal amount of either NanoJuice component is at the lowest or highest end of the range, test further to extend the range.
	Insufficient amount of transfection complex	Increase the amount of DNA. When increasing the amount of DNA, increase the amount of each NanoJuice Transfection Reagent proportionately.
	Cell density is suboptimal at time of transfection	Cell density guidelines are provided in Table 2. However, optimal cell density should be determined for each cell type. Try higher and lower cell densities.
	Serum present during the transfection protocol	Use only serum-free medium during formation of transfection complexes.
		If cells were grown in presence of serum, wash cells once before adding serum-free medium and then add the transfection mix.
	Poor quality DNA	Prepare fresh endotoxin-free plasmid DNA. Include an endotoxin removal step in your plasmid preparation protocol. Alternatively, prepare super-coiled plasmid DNA using a CsCl/EtBr protocol.
	Harvesting period is suboptimal	Harvesting periods are dependent on the cell line and the gene being expressed. Most cell lines exhibit maximal expression for most genes between 24–48 hours post transfection. If the time period for maximal expression for a particular cell line and gene in question is not known, perform a time course experiment to determine the optimal harvesting time.
High cell toxicity	Excessive amount of NanoJuice reagents/DNA in transfection	Reduce the amount of transfection mixture used per dish while keeping the ratios of the Nanojuice reagents to DNA constant.
		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.

	Antibiotics present during transfection	Increased cell permeability during transfection causes high antibiotic influx, thereby harming cell growth. Avoid using antibiotics during transfection.