RiboJuice™ siRNA Transfection Reagent

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

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
UK Freephone 0800 622935
Ireland Toll Free 1800 409445
customer.service@
merckbiosciences.co.uk

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

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www.novagen.com

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Description

RiboJuice™ siRNA Transfection Reagent	0.3 ml	71115-3
	1.0 ml	71115-4

RNA interference experiments using siRNA require a transfection reagent specifically optimized for siRNA delivery. RiboJuice siRNA Transfection Reagent efficiently delivers small interfering RNA (siRNA) into a wide range of mammalian cell lines for targeted gene suppression (1). When annealed to the target mRNA in the cell, the siRNA directs cleavage of the target message resulting in suppression of protein expression without affecting non-target genes (2–6). Successful siRNA experiments require well designed siRNA and a highly efficient delivery method into healthy eukaryotic cells. RiboJuice is an optimized easy-to-use siRNA transfection reagent formulated for minimal cell toxicity. In addition, RiboJuice is compatible with GeneJuice™ Transfection Reagent (Cat. No. 70967) for co-transfection of siRNA and plasmid DNA, providing a mechanism for normalizing transfection efficiency with a reporter gene (1).

Cell lines successfully transfected with RiboJuice siRNA Transfection Reagent					
A549	BHK	CHO	COS-7	HEK293	HeLa
HepG2	HONE-1	L428	L591	L6	MCF-7
Neuro-2A	NIH 3T3				
Primary or first passage rat HSC		Primary aortic smooth muscle		Primary keratinocytes	

Components

0.3 or 1 ml RiboJuice siRNA Transfection Reagent (2.5 mg/ml in 100% ethanol)

Storage

Store RiboJuice tightly capped at 4°C.

Design of siRNA

Many guidelines for siRNA design have originated from the Thomas Tuschl lab at the Max-Planck Institute for Biophysical Chemistry. siRNA target sequence should be positioned at least 50 to 100 nucleotides downstream of the translational start site to avoid regulatory proteins. The sequence content should be roughly 50% G/C and the sense strand should have sequence structure of $AA(N_{19})TT$ or $AA(N_{21})$. Candidate siRNAs should be analyzed using a search of the NCBI database (http://www.ncbi.nlm.nih.gov) to ensure that only one gene is targeted by a particular siRNA. Several candidates should be tested to evaluate their efficiencies (7, 8). Store siRNA at –70°C and dilute in 100 mM NaCl., 50 mM Tris-HCl, pH 8.

General Transfection Considerations and Optimization

The following protocols are suitable for a range of cell types, but highest transfection efficiency may require optimization for each cell type and siRNA sequence. Use the tables below as a guideline. The most important variables are cell density, and amount of RiboJuice™ siRNA Transfection Reagent. Factors influencing the success of siRNA experiments include:

- Cell density: The cell line should be passaged regularly (every 2–3 days) to ensure rapid growth. Optimum cell density is normally between 50–80% confluency for adherent cells and 1.0–2.0 × 10^6 cells/ml for suspension cells. Optimal cell density should be determined empirically for each cell line, and kept constant for all experiments to ensure reproducibility.
- RiboJuice siRNA Transfection Reagent: To begin, use amount of RiboJuice recommended in table below. Optimize transfection efficiency by varying amount of RiboJuice Reagent 1 µl at a time (1–4 µl total per well of a 24-well plate), while maintaining the same concentration of siRNA.
- siRNA concentration: siRNA is generally effective between 0.1–25 nM final concentration. Start with a final concentration of 5 nM siRNA. The final concentration is calculated using the total volume of liquid per well. In a 24-well plate (siRNA transfection only), each well contains 250 µl medium plus 50 µl transfection mixture for a total volume of 300 µl. In a 24-well plate (co-transfection of siRNA and DNA) each well contains 250 µl medium plus 50 µl GeneJuice™ transfection mixture plus 50 µl RiboJuice transfection mixture for a total volume of 350 µl. The volume of siRNA should not exceed 10% of the transfection mix volume. Use the tables below, or see page 7 for an example calculation.
- Duration of transfection: Maximum target gene suppression from siRNA is typically observed between 24 and 72 h post-transfection. Supplement with additional growth medium after 24 h.

Transfection of siRNA	**Plate format (well/plate)**			**Dish format (mm)**		
Tissue Culture Format	**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)	2–4	4–8	10–20	10–20	28–56	80–160
Volume of complete growth medium in the well or dish (ml)	0.25	0.5	1.25	1.25	3.5	10
Volume of serum-free medium in the transfection mixture (µl)	48	96	244	244	683	1,950
Volume of RiboJuice siRNA Transfection Reagent (µl)	2	4	6	6	17	50
Volume of 1 µM siRNA stock (5 nM final concentration; µl)	1.5	3	7.5	7.5	21	60

Co-transfection of siRNA and Plasmid DNA	**Plate format (well/plate)**			**Dish format (mm)**		
Tissue Culture Format	**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)	2–4	4–8	10–20	10–20	28–56	80–160
Volume of complete growth medium in the well or dish (ml)	0.25	0.5	3	3	5	12
Volume of serum-free medium in the RiboJuice transfection (µl)	48	96	244	244	683	1,950
Volume of RiboJuice siRNA Transfection Reagent (µl)	2	4	6	6	17	50
Volume of 1 µM siRNA stock (5 nM final concentration; µl)	1.75	3.5	17.5	17.5	32	80
Volume of serum-free medium in the GeneJuice® transfection mixture (µl)	49	98	249	249	696.5	1,990
Volume of GeneJuice Transfection Reagent (µl)	1	2	5	5	14	40
Amount of plasmid DNA (µg)	0.25	0.5	1.25	1.25	3.5	10

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

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
UK Freephone 0800 622935
Ireland Toll Free 1800 409445
customer.service@
merckbiosciences.co.uk

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Transfection Procedure (24-well plate format)

The following procedures describe methods for introduction of siRNA into adherent and suspension cell cultures in a 24-well format. Alternative formats require adjusting the amount of RiboJuice™ siRNA Transfection Reagent and cell seeding densities, as described on page 3. A negative control (no siRNA) is recommended.

Cell preparation

The transfection protocol is identical for adherent or suspension cells except for cell plating. Seed the cells the day prior to transfection according to the following recommendations:

Adherent cells: Plate 2–8 × 10^4 cells per well of a 24-well plate in 250 µl of complete growth medium. Incubate at 37°C (5% CO_2) overnight. Cells should be 50–80% confluent before transfection.

Suspension cells: Dilute the cells to a density of 0.5–2.5 × 10^5 cells per ml. Incubate the cells at 37°C (5% CO_2) overnight. Cells should be in log phase growth before transfection. Plate cells at 2–4 × 10^5 cells per 250 µl in each well of a 24-well plate.

Transfection

Note: Medium containing polyanions (e.g. heparin) should not be used.

1. Combine 48 µl serum-free medium (e.g. GIBCO RPMI 1640 or Opti-MEM®) and 2 µl RiboJuice siRNA Transfection Reagent for each well/plate in a sterile tube. Volumes can be scaled up for transfection of multiple wells with the same siRNA.

Note: For most cell lines, the recommended amount of RiboJuice siRNA Transfection Reagent is 2 µl per well, but 1–4 µl may be used.

2. Mix thoroughly by gentle vortexing.
3. Incubate at room temperature for 5 min.
4. Add siRNA to RiboJuice/medium mixture and mix gently.

Note: The optimal amount of siRNA should be determined empirically. A final concentration of 0.1–25 nM is recommended.

5. Incubate siRNA/RiboJuice/medium mixture at room temperature for 5–15 min.
6. Add appropriate volume siRNA/RiboJuice/medium mixture (approximately 50 µl) dropwise to each well. Distribute drops evenly over medium in wells. Gently rock plate to ensure uniform distribution. Do not swirl plate because this action concentrates transfection mixture in center of plate. Final volume in well is 300 µl (250 µl complete medium + 50 µl transfection mixture).

Optional: Remove transfection mixture after 2–8 h and replace with complete growth medium.

7. Incubate cells for 24–72 h at 37°C (5% CO_2).
8. Harvest cells for characterization or reporter assays. CytoBuster™ Protein Extraction Reagent (Cat. No. 71009) efficiently extracts soluble protein from mammalian cells without need for secondary treatment (e.g. sonication, freeze/thaw). To assay reporter gene activity from a stable cell line incorporating a reporter gene, Reportasol™ Extraction Buffer (Cat. No. 70909) is formulated to extract and maintain maximal activity of reporter enzymes.

Co-transfection of siRNA and Plasmid DNA in a 24-well Plate Format

GeneJuice™ Transfection Reagent is compatible with RiboJuice™ siRNA Transfection Reagent for co-transfection of plasmid DNA and siRNA using the following protocol. Negative controls (no siRNA, or no plasmid DNA) are recommended.

Cell preparation

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.

Adherent cells: Plate 2–8 × 10^4 cells per well of 24-well plate in 250 µl complete growth medium. Incubate at 37°C (5% $C0_2$) overnight. Cells should be 50–80% confluent before transfection.
Suspension cells: Dilute cells to a density of 0.5–2.5 × 10^5 cells per ml. Incubate cells at 37°C (5% CO_2) overnight. Cells should be in log phase growth before transfection.

Transfection

Note: Medium containing polyanions (e.g. heparin) should not be used.

1. Prepare two transfection mixtures:

GeneJuice Transfection Reagent/plasmid DNA transfection mixture

a) Combine 1 µl GeneJuice with 49 µl serum-free medium (e.g. GIBCO RPMI 1640 or Opti-MEM®) in sterile tube for each well to be transfected.

b) Mix thoroughly by gentle vortexing.

c) Incubate at room temperature for 5 min.

d) Add 0.25 µg supercoiled plasmid DNA.

e) Incubate for 5–15 min at room temperature.

RiboJuice siRNA Transfection Reagent/siRNA transfection mixture

a) Combine 2 µl RiboJuice with 48 µl serum free medium (e.g. GIBCO RPMI 1640 or Opti-MEM) in sterile tube for each well to be transfected.

b) Mix thoroughly by gentle vortexing.

c) Incubate at room temperature for 5 min.

d) Add siRNA to RiboJuice/medium mixture and mix gently.

Note: Determine optimal amount siRNA empirically. A final concentration of 0.1–25 nM is recommended based on total volume in well, including additional volume from GeneJuice reaction.

e) Incubate for 5–15 min at room temperature.

2. Add both transfection mixtures (50 µl each) dropwise to each well. Distribute drops over medium in wells and gently rock plate to ensure uniform distribution. Do not swirl plate because this action concentrates transfection mixtures in center of plate. Final volume in well is 350 µl (250 µl complete medium + 50 µl GeneJuice transfection mixture + 50 µl RiboJuice transfection mixture).
3. Incubate cells for 24–72 h at 37°C (5% CO_2).
4. Harvest cells for characterization or reporter assays. Reportasol™ Extraction Buffer (Cat. No. 70909) efficiently extracts soluble reporter enzymes (e.g. *Renilla* luciferase, and β-galactosidase) from mammalian cells, while maintaining maximal activity. CytoBuster™ Protein Extraction Reagent (Cat. No. 71009) extracts cytoplasmic protein from mammalian cells.

Troubleshooting

Symptom	Possible cause	Solution
Minimal gene silencing	Serum present during formation of RiboJuice™/siRNA complex	Use only serum-free medium during formation of complex.
	Cell density sub-optimal at time of transfection	Optimal cell density should be determined for each cell type. Evaluate higher and lower cell densities.
	Ratio of RiboJuice to siRNA is sub-optimal	Titrate RiboJuice Reagent from 1–4 µl while maintaining constant siRNA level.
	Concentration siRNA is incorrect	Confirm concentration of siRNA and ensure final concentration is based on total volume in well. Vary siRNA concentration between 0.1 and 25 nM.
	Poor quality siRNA	Use fresh siRNA. siRNA should be stored as concentrated stocks at –80°C and diluted in 50 mM Tris-HCl pH 8, 100 mM NaCl as necessary.
	Ineffective siRNA	Design additional siRNAs to gene of interest, targeting alternate regions of the gene.
	Heparin or other polyanions in medium.	Use medium without polyanions.
Cell toxicity	Incomplete mixing of RiboJuice/siRNA complexes with cells	Thoroughly distribute transfection mixture to all cells in plate. Transfection mix should be added dropwise all across surface of medium. Plate should be rocked back and forth to mix. Do not swirl or rotate dish, as this may concentrate RiboJuice siRNA Transfection Reagent/siRNA complexes in center of dish.
	Excessive amount of RiboJuice/siRNA in transfection	Reduce amount of RiboJuice used per dish. Try removing transfection mixture after 2–8 h incubation, and replace with complete growth medium.
	Cell density too low at time of transfection	Cell density at time of transfection time is dependent upon initial plating density, cell growth rate (doubling time) and length of time between plating and transfection. Try plating more cells to achieve 50-80% confluency at time of transfection.
	Cell density too high at time of transfection	Cell density at time of transfection time is dependent upon initial plating density, cell growth rate (doubling time) and length of time between plating and transfection. Plate cells at a lower density to achieve 50-80% confluency at time of transfection. If cells are harvested at 72 h, additional complete medium may be added after 24 h to ensure cell viability.
	siRNA is targeting an essential gene causing a toxic effect	Include a negative control. One well or plate should omit siRNA. Compare wells or plates and evaluate if effect is due to presence of siRNA.

Guidelines for siRNA Quantification and Transfection

Quantify siRNA

Use the following guidelines to quantify siRNA.

1. Determine concentration of siRNA. Dilute sample of siRNA 1:25 and measure the A_{260} against blank. Use 100 mM NaCl, 50 mM Tris-HCl, pH 8, for blank and for siRNA dilutions. One A_{260} unit of siRNA = 40 µg/ml.

   Example: The A_{260} of a 1:25 dilution = 0.5.
   Therefore, the concentration is 0.5 × 40 µg/ml × 25 (Dilution Factor) = 500 µg/ml
2. Determine molar concentration (µg/nmol) of siRNA stock. The average MW of a nucleotide is 0.333 µg/ml.
   a) Number nucleotides (nt) × 2 strands = total number nt × 0.333 µg/nmol = × µg/nmol.
      Example of 23 nt siRNA: 23 × 2 = 46 × 0.333 µg/nmol= 15.3 µg/nmol
   b) Divide concentration of siRNA in µg/ml by number of µg/nmol to determine siRNA molar concentration (µM).
      Example: $\frac{500\ \mu g/ml}{15.3\ \mu g/nmol}$ = 32.6 nmol/ml or µM
3. Dilute siRNA stock to 1 µM with 100 mM NaCl, 50 mM Tris-HCl, pH 8.
4. Add siRNA to transfection according to table on page 3, or as determined below.

Determine volume of 1 µM siRNA stock to add per transfection

Use the following calculations, or refer to the tables on page 3 to determine the volume of siRNA to use per transfection. In the example calculation, the desired final concentration of siRNA is 25 nM. The volume of medium per well (of 24-well plate) is 0.25 ml plus 50 µl of transfection mix (47 µl medium + 3 µl RiboJuice™) for a total liquid volume per well of 300 µl.

$\frac{\text{desired siRNA concentration}}{\text{siRNA stock concentration}}$ × total volume per well/dish = volume of stock siRNA to add

$\frac{0.025\ \mu M}{1\ \mu M} \times 300\ \mu l = 7.5\ \mu l$

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