SKU # SL100570 Store at 4 °C

GenJet™ siRNA Transfection Reagent

----- A General Protocol for Transfecting siRNA to Mammalian Cells

☐ 100 µL
☐ 500 µL
☐ 1000 µL

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This product is for laboratory research ONLY and not for diagnostic use

INTRODUCTION

GenJet™ siRNA Transfection Reagent is a proprietary hybrid liposome–polymer formulation designed for highly efficient siRNA delivery with minimal cytotoxicity. Built upon our advanced lipid-conjugation and polymer synthesis technologies, GenJet™ siRNA Reagent provides robust gene silencing performance and offers significant advantages over conventional siRNA transfection reagents, including enhanced transfection efficiency, improved cell viability, and broad compatibility across a wide range of mammalian cell types.

IMPORTANT GUIDELINES FOR TRANSFECTION

Buffer requirement: GenJet™ Transfection Buffer working solution (1 x) must be used to dilute both siRNA/DNA and GenJet™ Reagent. Use of alternative diluents is not recommended, as buffer composition directly affects lipopolyplex formation and transfection efficacy.

Protocol optimization:

Standard protocols for siRNA transfection and siRNA/DNA co-transfection are provided below. As cell type, passage number, and target gene expression level can significantly influence outcomes, empirical optimization of reagent-to-nucleic-acid ratios, cell seeding density, and transfection duration is strongly advised to achieve maximal gene silencing.

PART I. Standard siRNA Transfection of Adherent Cells

Step I. Preparation of Working Solution of GenJet™ Transfection Buffer (1x):

GenJet™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution (1x), dilute one part of the stock solution with 4 parts of ddH_2O into a sterile bottle. The working solution is stable at RT for 24 months.

Note: *Always keep GenJet™ Transfection Buffer (5x) at RT. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After dilution with 4 parts of ddH_2O to make GenJet™ Transfection Buffer (1x) working solution, the white precipitates will disappear. Always keep GenJet™ Transfection Buffer working solution (1x) at RT.*

Step II. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~50% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 min before transfection.

Note: *GenJet™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.*

Table 1. A Guideline for siRNA transfection per cell culture vessel

Culture Dish	Growth Medium (mL)	Transfection Buffer (µL)	siRNA (pmol) Final 5.0 or 50 nM	GenJet™ Reagent (µL)
24-well	1.0	50	5.0 / **50**	1.2 ~ **2.0**
12-well	1.5	75	7.5 / **75**	2.0 ~ **3.3**
6-well	2.0	100	10 / **100**	2.4 ~ **4.0**
60 mm	5.0	300	30 / **300**	7.2 ~ **12**
10 cm / Flask 75	10	600	50 / **500**	12 ~ **24**

Step III. siRNA Transfection Protocol:

For optimal siRNA-mediated silencing, we recommend using 1~100 nM siRNA. As a starting point, we recommend using 5.0 nM siRNA which usually gives satisfactory silencing result for most adherent cell lines or primary cells. For hard-to-transfection cells, we recommend using a final siRNA concentration of 50 nM. (bold & underlined in **Table 1**).

The following conditions are given per well in a 6 well plate. For other culture format, please refer to **Table 1**.

- For each well, add 2.0 mL of complete medium with serum and antibiotics freshly 30~60 min before transfection.
- Dilute 10 or **100** pmol siRNA (final concentration of 5.0 or **50** nM respectively per well) into 100 µL of working solution of GenJet™ Transfection Buffer prepared in **Step I**. Vortex to mix.

Note: *For maximum gene silencing, dilute siRNA and GenJet™ reagent with GenJet™ Transfection Buffer working solution (1x). We strongly suggest reconstituting siRNA stock solution at 10 µM, so add 1.0 or 10 µL siRNA stock solution per well of 6-well plate to make final 5.0 and 50 nM siRNA respectively.*

- Add 2.4 µL or **4.0 µL** (for hard-to-transfect cells, bold and underlined in **Table 1**) GenJet™ reagent, mix by pipetting up and down.
- Incubate for ~15 min at RT to let transfection complex form. **Note: Never keep the complex longer than 30 min.**
- Add the transfection mix to the cells drop wise. Gently rock the plate back and forth and return the plate to CO2 incubator.
- Replace transfection medium by cell growth medium ~5 hours after transfection when necessary.
- Gene silencing is usually measured 24~72 hours post transfection.

PART II. A Standard Protocol for DNA/siRNA Co-transfection

Step I. Preparation of Working Solution of GenJet™ Transfection Buffer:

GenJet™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution (1x), dilute one part of the stock solution with 4 parts of ddH_2O into a

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sterile bottle. The working solution is stable at RT for 24 months.

Note: *Always keep GenJet™ Transfection Buffer (5x) at RT. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After dilution with 4 parts of ddH_2O to make GenJet™ Transfection Buffer (1x) working solution, the white precipitates will disappear. Always keep GenJet™ Transfection Buffer working solution (1x) at RT.*

Step II. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~70% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 min before transfection.

Note: GenJet™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.

Table 2. A Guideline for DNA & siRNA Co-transfection Per Cell Culture Vessel

Culture Dish	Growth Medium (mL)	Transfection Buffer (µL)	Plasmid DNA (µg)	siRNA (pmol) Final 10 nM	GenJet Reagent (µL)
24-well	1.0	50	0.25	10	1.5
12-well	1.5	75	0.375	15	2.25
6-well	2.0	100	0.5	20	3
60 mm	5.0	300	1.5	50	7.5
10 cm / flask 75	10	600	4.0	100	15

Step III. DNA & siRNA co-transfection protocol:

For DNA/siRNA co-transfection experiment, we recommend using 0.3~0.5 µg DNA and 1~20 nM siRNA per well in a 6-well plate. As a starting point, we recommend using 0.5 µg DNA and 10 pmol siRNA (final concentration 10 nM) per well of a 6-well plate which usually give satisfactory knockdown effect.

The following conditions are given per well of a 6 well plate. For other culture format, please refer to **Table 2**.

- For each well, add 1.0 mL of complete medium with serum and antibiotics freshly 30~60 min before transfection.
- Dilute 0.5 µg DNA and 20 pmol siRNA (final 10 nM) into 100 µL of working solution of GenJet™ Transfection Buffer prepared from **Step I**. Mix by pipetting up and down.

  Note: *For optimal transfection efficiency and maximum gene silencing, GenJet™ Transfection Buffer working solution is a must for diluting siRNA/DNA and GenJet™ reagent. We strongly suggest preparing siRNA stock solution at 10 µM, so add 2.0 µL siRNA stock solution per well of 6-well plate to make final 10 nM of siRNA.*
- Add 3 µL GenJet™ reagent immediately, mix by pipetting up and down.
- Incubate for ~15 min at RT to let transfection complex form.

Note*: Never keep the complex longer than 30 min.*

- Add the transfection complex to the cells drop wise.
- Gently rock the plate back and forth and return the plate to the incubator.
- Replace transfection medium by cell growth medium ~5 hours after transfection when necessary.
- Gene silencing is usually measured 24~72 hours post transfection.

Important: *GenJet siRNA Transfection Reagent differs significantly from GenJet DNA Transfection Reagent in both chemistry and formulation. Please ensure you purchase the appropriate reagent for your intended application.*

Storage: GenJet™ siRNA Transfection Reagent is stable for up to 24 months at 4 °C. Always keep GenJet™ Transfection Buffer (5x) at RT. This item is shipped at ambient temperature.