

LipoJet™ In Vitro Transfection Kit

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



10075 Tyler Place, Suite 19
Ijamsville, MD 21754
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagen.com
Web: www.signagen.com

- 100 µl
- 500 µl
- 1000 µl

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

Introduction:

LipoJet™ In Vitro Transfection Kit was formulated by our innovative and proprietary lipid-conjugation technology. LipoJet™ Transfection Kit exhibits significant difference from other lipids transfection reagents in the market. LipoJet™ Transfection Kit is the most powerful gene delivery tool for a variety of applications including plasmid DNA, siRNA, and shRNA delivery.

Important Guidelines for Transfection:

- LipoJet™ reagent was formulated as a versatile gene delivery tool. While this protocol gives procedures for transfecting siRNA and co-transfecting DNA/siRNA to mammalian cells, the protocol for DNA transfection can be obtained from our website by visiting <http://signagen.com/lipojet>
- For maximum silencing efficiency, we recommend using LipoJet™ Transfection Buffer to dilute DNA, siRNA and LipoJet™ Reagent.

PART I. Standard siRNA Transfection of Adherent Cells.

Step I. Preparation of Working Solution of LipoJet™ Transfection Buffer

LipoJet™ Transfection Buffer (5x) is provided as 5 times concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH₂O to a sterile bottle. The working solution is stable at 4 °C~RT for 12 months.

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 minutes before transfection.

Note: LipoJet™ 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 (pmols) Final 5.0 nM	LipoJet™ (µL)
24-well	0.5	50	2.5	0.75
12-well	0.75	75	3.75	1.2
6-well	1.0	100	5.0	1.5
60 mm	3.0	300	15	4.5
10 cm / Flask 75	8.0	800	40	12

Step III. siRNA Transfection Protocol

For optimal siRNA-mediated silencing, we recommend using 5~20 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 10 nM. Due to the exceptional siRNA condensing capacity of LipoJet™ reagent, we recommend using same amount of LipoJet™ reagent for final 5~20 nM of siRNA (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 1.0 ml of complete medium with serum and antibiotics freshly 30 ~ 60 minutes before transfection.
- Dilute 5.0 pmols siRNA (final concentration of 5.0 nM respectively per well) into 100 µl working solution of LipoJet™ Transfection Buffer. Mix by pipetting up and down.

Note: For maximum gene silencing, using LipoJet™ Transfection Buffer to dilute siRNA and LipoJet reagent is a must.

We strongly suggest reconstituting siRNA stock solution at 5.0 µM, so add 1.0 µl siRNA stock solution per well of 6-well plate to make final 5.0 nM siRNA.

- Add 1.5 µl LipoJet™ reagent, mix by pipetting up and down.
- Incubate for 10~15 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.
- Gene silencing is usually measured 24~72 hours post transfection for mRNA levels and 48~96 hours for proteins.

Storage: LipoJet™ In Vitro Transfection Kit is stable for up to 12 months at 4 °C. This item shipped at ambient temperature

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

Step I. Preparation of Working Solution of LipoJet™ Transfection Buffer

LipoJet™ Transfection Buffer (5x) is provided as 5 times concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH₂O to a sterile bottle. The working solution is stable at 4 °C~RT for 12 months.

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 minutes before transfection.

Note: LipoJet™ 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 (pmols) Final 5.0 nM	LipoJet™ (µL)
24-well	0.5	50	0.25	2.5	1.5
12-well	0.75	75	0.375	3.25	2.25
6-well	1.0	100	0.5	5.0	3
60 mm	3.0	300	1.5	15	9
10 cm / flask 75	8.0	800	4.0	40	24

Step III. DNA & siRNA co-transfection protocol:

For DNA/siRNA co-transfection experiment, we recommend using 0.3~0.5 µg DNA and 5~20 nM siRNA per well in a 6-well plate. As a starting point, we recommend using 0.5 µg DNA and 5.0 pmols siRNA (final concentration 5.0 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 minutes before transfection.
- Dilute 0.5 µg DNA and 5.0 pmols siRNA (final 5.0 nM) into 100 µl of LipoJet™ Transfection Buffer. Mix by pipetting up and down.

Note: For maximum gene silencing, using LipoJet™ Transfection Buffer to dilute siRNA/DNA and LipoJet™ reagent is a must. We strongly suggest preparing siRNA stock solution

at 5.0 µM, so add 1.0 µl siRNA stock solution per well of 6-well plate to make final 5.0 nM of siRNA.

- Add 3 µl LipoJet™ reagent immediately, mix by pipetting up and down.
- Incubate for 10~15 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 4~5 hours after transfection when necessary.
- Gene silencing is usually measured 24~48 hours post transfection for mRNA levels and 48~72 hours for proteins.

Storage: LipoJet™ In Vitro Transfection Kit is stable for up to 12 months at 4 °C. This item shipped at ambient temperature