Cat # SL100468 Store at 4 °C

LipoJet™ In Vitro DNA and siRNA Transfection Kit (Ver. II)

----- A General Protocol for Transfecting Mammalian Cell

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
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This product is for laboratory research ONLY and not for diagnostic use

Introduction:

Based on our innovative and proprietary lipid-conjugation technology, LipoJet™ Transfection Kit, formulated from novel fluorinated cationic lipids, exhibits significant difference from other lipids transfection reagents in the market. LipoJet™ Transfection Kit is the most powerful yet very gentle gene delivery tool for a variety of applications including plasmid DNA and/or siRNA for most of mammalian cell types. Compared with leading products in the market, LipoJet™ is more cost-effective and always provides higher transfection efficiency with less cytotoxicity.

Contents Per Kit:

1. 1 x 1.0 ml of LipoJet™ DNA In Vitro Transfection Reagent
2. 1 x 8.0 ml of LipoJet™ Transfection Buffer (5x)

Important Guidelines for Transfection:

- LipoJet™ reagent was formulated for DNA and siRNA transfection. The following standard protocol is given for DNA and siRNA transfection to mammalian cells. For a protocol of siRNA/DNA co-transfection, please email us at info@signagen.com
- For better efficiency, choosing LipoJet™ Transfection Buffer (1x) is a must.
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics.

Part I. A General Protocol for DNA Transfection.

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

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

Note: Always keep LipoJet™ 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 LipoJet™ Transfection Buffer (1x) working solution, the white precipitates will disappear.

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 60~70% 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: High serum levels (>5%) with antibiotics do NOT have inhibitory effect on transfection efficiency. We recommend using complete serum/antibiotics-containing medium to grow the cells during transfection.

Step III. Preparation of LipoJet™ -DNA Complex and Transfection Procedures:

For different cell types, the optimal ratio of LipoJet™ (µL):DNA (µg) varies from 2:1 to 3:1. We recommend using LipoJet™ (µL):DNA (µg) at 2:1 at a starting point.

The following protocol is given for transfection in 24-well plates, refer to **Table 1** for transfection in other culture formats.

- For each well of 24-well plate, dilute 0.5 µg of DNA into 50 µl of LipoJet™ Transfection Buffer (1x) prepared from **Step I**. Mix by vortexing.
- Add 1.0 µl of LipoJet™ reagent, vortex briefly to mix.
- Incubate for ~10 min at RT to allow LipoJet™/DNA complex to form.

  Note: Never keep the LipoJet™/DNA complex longer than 20 min.
- Add the LipoJet™/DNA transfection mix to the cells in serum containing medium drop wise.
- Swirl plate gently to homogenize.
- Check transfection efficiency 24 to 48 hours post transfection. 48 hours usually give better efficiency.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium (ml)	Plasmid DNA (µg)	LipoJet™ Transfection Buffer (1x) (µL)	LipoJet™ Reagent (µL)
96-well	0.1	0.1	5	0.2 ~ 0.3
48-well	0.25	0.25	25	0.5 ~ 0.75
24-well	0.5	0.5	50	1 ~ 1.5
6-well	2	2.0	200	4 ~ 6
35 mm dish	2	2.0	200	4 ~ 6
60 mm dish	4	4.0	400	8 ~ 12
10 cm / T75	10	10	800	20 ~ 30
15 cm / T175	20	20	1600	40 ~ 60

Storage: LipoJet™ Reagent is stable for up to 12 months at +4 °C after receipt

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LipoJet™ In Vitro DNA and siRNA Transfection Kit

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- ☐ 100 µl
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Part II. A General Protocol for siRNA Transfection.

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

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

Note: Always keep LipoJet™ 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 LipoJet™ Transfection Buffer (1x) working solution, the white precipitates will disappear.

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 time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. We recommend using complete serum/antibiotics-containing medium to grow the cells during transfection.

Step III. Preparation of LipoJet™-siRNA Complex and Transfection Procedures:

For optimal siRNA-mediated silencing, we recommend using 10~80 nM siRNA (final concentration). The following protocol is given for transfection in 6-well plate, refer to **Table 2** for transfection in other culture formats.

- For each well, dilute 20 ~ 160 pmoles siRNA (for a final concentration of 10 to 80 nM per well) into 200 µl of LipoJet™ Transfection Buffer (1x) prepared from **Step I**. Mix gently.
- Add 4 µl of LipoJet™ reagent, vortex briefly to mix.
- Incubate for ~10 min at RT to allow LipoJet™/siRNA complexes to form.

  Note: Never keep the LipoJet™/siRNA complex longer than 20 min.
- Add the LipoJet™/siRNA transfection mix to the cells in serum-containing medium drop wise.
- Swirl plate gently to homogenize.
- Check siRNA silencing efficiency 24 to 72 hours post transfection. 48~72 hours usually give better efficiency.

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium (ml)	siRNA (pmoles) 10~80 nM	LipoJet™ Transfection Buffer (1x) (µL)	LipoJet™ Reagent (µL)
96-well	0.1	1 ~ 8	10	0.4
48-well	0.25	2.5 ~ 20	25	1
24-well	0.5	5 ~ 40	50	2
6-well	2	20 ~ 160	200	4
35 mm dish	2	20 ~ 160	200	4
60 mm dish	4	40 ~ 320	400	8
10 cm / T75	10	100 ~ 800	800	20

Storage: LipoJet™ Reagent is stable for up to 12 months at +4 °C after receipt