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LiFectRNA™ Transfection Reagent

Cat. #: M0141 Size: 1 ml

Introduction:

LiFectRNA™ Transfection Reagent is a novel biodegradable polymer based siRNA and DNA transfection reagent. With our proprietary pH Dependent Conformational Change (PDCC) technology, the polymer was chemically modified by addition of pre-screened hydrophobic groups to side chain, making LiFectRNA™ Reagent a versatile and most powerful gene delivery tool. LiFectRNA™ Reagent have been validated to effectively and reproducibly transfect single siRNA, miRNA mimics, DNA or co-transfect DNA/siRNA, DNA/miRNA mimics to variety of mammalian cells.

Important Guidelines for Transfection

- For maximum gene silencing, using LiFectRNA™ Transfection Buffer working solution (1×) to dilute siRNA/DNA and LiFectRNA™ Reagent is a must.
- While the standard protocols for siRNA transfection and siRNA/DNA co-transfection are being given below, optimization is often needed for maximal gene silencing.

Part I. Standard siRNA Transfection of Adherent Cells

Step I. Preparation of Working Solution of LiFectRNA™ Transfection Buffer (1×)

LiFectRNA™ Transfection Buffer (5×) is provided as 5× concentrated stock solution. To make working solution (1×), 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 LiFectRNA™ Transfection Buffer (5×) 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 LiFectRNA™ Transfection Buffer (1×) working solution, the white precipitates will disappear. Always keep LiFectRNA™ Transfection Buffer working solution (1×) 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: LiFectRNA™ 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 (pmoles) Final 5.0 or 50 nM	LiFectRNA™ Reagent (µl)
24-well	0.5	50	2.5 / 25	1.2-2.0
12-well	0.75	75	3.75 / 38	2.0-3.3
6-well	1.0	100	5.0 / 50	2.4-4.0
60 mm	3.0	300	15 / 150	7.2-12.0
10 cm / Flask 75	2.8	800	40 / 400	20-33.0

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 1.0 ml of complete medium with serum and antibiotics freshly 30~60 min before transfection.
- Dilute 5.0 or 50 pmoles siRNA (final concentration of 5.0 or 50 nM respectively per well) into 100 µl of working solution of LiFectRNA™ Transfection Buffer prepared in Step I. Vortex to mix.

Note: For maximum gene silencing, dilute siRNA and LiFectRNA™ reagent with LiFectRNA™ Transfection Buffer working solution (1×).

We strongly suggest reconstituting siRNA stock solution at 10 µM, so add 0.5 or 5.0 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**) LiFectRNA™ 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 CO_2 incubator.
- Replace transfection medium by cell growth medium ~5 hours after transfection when necessary.
- Gene silencing is usually measured 24~72 hours post transfection.

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

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

LiFectRNA™ Transfection Buffer (5×) is provided as 5× concentrated stock solution. To make working solution (1×), dilute one part of the stock solution with 4 parts of ddH$_2$O into a sterile bottle. The working solution is stable at RT for 24 months.

Note: Always keep LiFectRNA™ Transfection Buffer (5×) at RT. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After dilution with 4 parts of ddH$_2$O to make LiFectRNA™ Transfection Buffer (1×) working solution, the white precipitates will disappear. Always keep LiFectRNA™ Transfection Buffer working solution (1×) 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: LiFectRNA™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.

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 pmoles 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 10 pmoles siRNA (final 10 nM) into 100 µl of working solution of LiFectRNA™ Transfection Buffer prepared from Step I. Mix by pipetting up and down.

Note: For optimal transfection efficiency and maximum gene silencing, LiFectRNA™ Transfection Buffer working solution is a must for diluting siRNA/DNA and LiFectRNA™ reagent. We strongly suggest preparing siRNA stock solution at 10 µM, so add 1.0 µl siRNA stock solution per well of 6-well plate to make final 10 nM of siRNA.

- Add 3 µl LiFectRNA™ reagent immediately, mix by pipetting up and down.
- Incubate for ~15 min at RT to let transfection complex form.

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 10 nM	LiFectRNA™ Reagent (µl)
24-well	0.5	50	0.25	5	1.5
12-well	0.75	75	0.375	7.5	2.25
6-well	1.0	100	0.5	10	3
60 mm	3.0	300	1.5	30	9
10 cm / Flask 75	2.8	800	4.0	80	24

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.

Storage: LiFectRNA™ Transfection Reagent is stable for up to 12 months at 4°C. Always keep LiFectRNA™ Transfection Buffer (5×) at RT. This item is shipped at ambient temperature.

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