Lip3000SSS Transfection Reagent

Features:

◎Excellent cell transfection performance: Lip3000SSS can transfect DNA into various adherent cells. The positive rate of transfection in adherent cells can be as high as over 90%;

◎Powerful transfection function: Lip3000SSS can not only efficiently transfect large molecular plasmid DNA, but also mRNA, siRNA, mimics and various small molecular DNA;

◎Extremely low cytotoxicity: Lip3000SSS is formulated with new degradable nanomaterials, which has very low cytotoxicity. The death rate of transfected cells is only about 10%, which greatly reduces the impact of cytotoxicity on experimental results.

◎Easy to operate: Lip3000SSS can be used for transfection of cells cultured in serum-containing medium, and there is no need to replace the culture medium before or after transfection.

Components

Cat. No.	Lip3000SSS	Buffer A	Trans buffer S
OM750040-0.1ml	0.1ml	80ul	3ml
OM750040-0.75ml	0.75ml	0.6ml	22.5ml
OM750040-1.5ml	1.5ml	1.2ml	45ml

Introduction:

Lip3000SSS Transfection Reagent is a kit specially developed for efficient nucleic acid transfection based on Rfect Plasmid Transfection Reagent.. It boasts excellent transfection performance, capable of efficiently transfecting most adherent cells with a transfection efficiency of over 90% (HeLa cells, EGFP plasmid). Lip3000SSS is highly versatile, as it can not only efficiently transfect large molecular plasmid DNA, but also mRNA, siRNA, mimics, and various small molecular DNA. Unlike common liposome transfection reagents available on the market, Lip3000SSS is formulated with biodegradable materials, resulting in extremely low cytotoxicity, with almost no significant cell death observed 24 hours after transfection. Lip3000SSS is also very easy to use: simply mix the transfection reagent with plasmid DNA or siRNA, then directly add the mixture to the cultured cells. Serum does not affect its transfection effect, eliminating the need for deliberate addition or replacement of the culture medium, making the operation extremely convenient. Lip3000SSS is suitable for nucleic acid transfection of adherent cells. For transfecting primary cells, please choose the Omni Prime Primary Cell Nucleic Acid Transfection Kit. For transfecting suspension cells, please select the Omni Prime Suspension Cell Nucleic Acid Transfection Kit.

Protocol:

Protocol 1　Plasmid DNA transfection (For 24-well plate transfection):

A. Cell seeding:

1. One day before transfection, 0.8-1.2×10^5 cells are seeded per well in growth medium without antibiotics so that cells will be 85% confluent at the time of transfection. Ensure that cells grow well (very important).
2. It is best to replace the culture medium with fresh serum-containing medium before transfection to prevent cell death due to excessive cell density and insufficient nutrition during the incubation period after transfection.

B. Prepare Lip3000SSS/DNA complex (transfection should be carried out immediately after this step):

1. Add 30μl of Trans buffer S to a 1.5 ml sterile centrifuge tube, then add an appropriate amount of transfection reagent as specified in the attached table 1. Gently mix with a pipette and incubate at room temperature for 5 minutes.
2. Add 30μl of Trans buffer S to a 1.5 ml sterile centrifuge tube, add an appropriate amount of Solution A, mix gently, then add an appropriate amount of DNA . See the attached table 1 for the specific amounts of Buffer A and DNA. Mix gently again with a pipette and incubate at room temperature for 5 minutest.
3. Add the DNA-Trans buffer S mixture into the RFect-Trans buffer S mixture, gently mix with a pipette, and incubate at room temperature for 15 minutes, then proceed with transfection immediately. Note: The order of mixing the RFect-Trans buffer S mixture and the DNA-Trans buffer S mixture is very important; do not reverse it.

   Note: Use polypropylene centrifuge tubes.

C. Transfection:

1. Add the transfection complex prepared in step B to the culture medium, and gently shake the culture plate while adding to evenly distribute the complex. After adding, immediately transfer the culture plate to the incubator for continued culture.
2. Observe after 12 hours of culture, and the best observation or harvest time is 24-48 hours.
3. Lip3000SSS still has a high transfection efficiency in complete culture medium, so there is no need to switch to serum-free or low-serum culture medium before and after transfection.

Protocol 2 siRNA transfection (For 24-well plate transfection):

A. Cell seeding:

1. One day before transfection, cells should be seeded per well in growth medium without antibiotics, and cells confluent should be between 30%-50% at the time of transfection. Ensure that cells grow well and are free of mycoplasma contamination (very important).
2. It is best to replace the growth medium with fresh serum-containing medium before transfection to prevent cell death due to excessive cell density and insufficient nutrition during the incubation period after transfection.

B. Prepare Lip3000SSS/siRNA complex (transfection should be carried out immediately after this step):

1. Add 30μl Trans buffer S to a 1.5 ml sterile centrifuge tube and add an appropriate amount of transfection reagent (see the table 2). Mix gently with a pipette and incubate at room temperature for 5 minutes.
2. Add 30μl Trans buffer S to a 1.5 ml sterile centrifuge tube and add an appropriate amount of siRNA (see the table 2). Mix gently with a pipette and incubate at room temperature for 5 minutes.
3. Add the siRNA-Trans buffer S mixture into the RFect-Trans buffer S mixture, mix gently with a pipette and incubated at room temperature for 15 minutes, and transfect immediately..

C. Transfection:

1. Add the transfection complex prepared in step B to the culture medium, gently shaking the culture plate while adding. After adding, immediately transfer the culture plate to the incubator for continued culture.
2. Culture at 37° C for 24-72 hours and detect the gene inhibition effect. If necessary, the culture medium can be changed after 4-6 hours of cell culture, but it is not necessary.
3. Lip3000SSS still has a high transfection efficiency in complete culture medium, so it is not necessary to change to serum-free or low-serum culture medium before and after transfection.

Important Guidelines for Transfection:

1. If you are conducting a transfection experiment for the first time, please be sure to perform an optimization experiment. For example, for 24-well plate plasmid transfection, the plasmid dosage per well is 0.8μg, Lip3000SSS can be optimized by using 2.0μl, 2.5μl,3.0μl or 3.5μl; For 24-well plate siRNA transfection, the Lip3000SSS dosage per well is 2μl, siRNA can be optimized by using 10pmol, 20pmol, 30pmol, 40pmol.
2. For plasmid transfection, the cell confluence should be between 80-90% at the time of transfection. Too high or too low cell confluence will affect the transfection efficiency. For siRNA transfection, the cell confluence should be between 30-50% at the time of transfection.
3. Buffer A is only used for plasmid transfection. It is not necessary to add Buffer A for RNA or siRNA transfection.
4. Please note the differences in cell density requirements for plasmid transfection and siRNA transfection, and do not use the same conditions.
5. For stable transfection, passage cells into selective medium 24-48 hours post-transfection.
6. This product is suitable for the independent transfection of plasmid DNA and siRNA respectively. For co-transfection of plasmid DNA and siRNA, please choose the Omni Prime Nucleic Acid Co-Transfection Kit.

Table 1: Recommended **Plasmid DNA** transfection conditions

Culture vessel		96-well	48-well	24-well	12-well	6-well	10cmdish
Vol. of Reagent	Trans buffer S (μl)	2x6	2x15	2x30	2x60	2x120	2x600
	Buffer A(μl)	0.4	1.0	2.0	4.0	8	40
	Lip3000SSS (μl)	0.5	1.3	2.5	5.0	10	50
	1μg/μl plasmid (μl)	0.16	0.4	0.8	1.6	3.2	16
Complete medium(ml)		0.10	0.25	0.50	1.0	2.0	10

Table 2: Recommended **siRNA** transfection conditions

Culture vessel		96-well	48-well	24-well	12-well	6-well	10cmdish
Vol. of Reagent	Trans buffer S (μl)	2x6	2x15	2x30	2x60	2x120	2x600
	Lip3000SSS (μl)	0.4	1.0	2.0	4.0	8.0	40
	siRNA (pmol)	4	10	20	40	80	400
Complete medium(ml)		0.10	0.25	0.50	1.0	2.0	10

Storage, Transportation, Stability and Special Handling:

Transport with ice packs. Store at 2-8 ℃. Keep in dark place. Valid for one year. Please mix reagents gently before use.

Reagent use and Limitations:

For Research Use Only. Not for use in diagnostic procedures.