Lip2000SSS Transfection Reagent

Features:

◎Excellent cell transfection performance: Lip2000SSS Prime can transfect DNA into most adherent cells and suspension cells. The positive rate of plasmid DNA transfection in some adherent cells can be as high as over 90%, and the positive rate of Fam-siRNA transfection can be as high as about 95%;

◎Ideal transfection efficiency can be obtained for many cells that are difficult to be transfected, such as neuronal cell line HT-22, N2A, microglial cell line BV2, macrophage cell line Raw264.7, leukemia cell line K562, colon cancer cell line HCT116, SW480 etc;

◎Extremely low cytotoxicity: Lip2000SSS Prime 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.

Components

Cat. No.	Lip2000 SSS	Buffer A	Trans buffer S
OM750041-0.3ml	0.3ml	0.6ml	12ml
OM750041-0.75ml	0.75ml	1.5ml	30ml
OM750041-1.5ml	1.5ml	3.0ml	60ml

Introduction:

Lip2000SSS Prime is an upgraded product of the Lip2000SSS plasmid DNA transfection reagent developed by the overseas returnee team of Changzhou Bio-generating Biotechnology Corp. It adds conformationally variable lipid molecules that can efficiently transfect siRNA/miRNA. Therefore, Lip2000SSS Prime can efficiently transfect not only plasmid DNA molecules, but also small molecule nucleic acids such as siRNA, miRNA, and inhibitor. Lip2000SSS Prime has very powerful transfection performance and can efficiently transfect DNA, siRNA, miRNA, etc. into most adherent cells and suspension cells. The transfection rate of plasmid DNA transfection in some adherent cells can be as high as over 90%, and the transfection rate of Fam-siRNA transfection can be as high as about 95%. Ideal transfection efficiency can also be obtained for many cells that are difficult to be transfected, such as neuronal cell line HT-22, N2A, microglial cell line BV2, macrophage cell line Raw264.7, leukemia cell line K562, colon cancer cell line HCT116, SW480, gastric cancer cell AGS etc.

Lip2000SSS Prime is formulated with biodegradable materials and has very low toxicity to cells. There is almost no obvious cell death 24 hours after transfection. This reagent is also very easy to use. First, mix the transfection reagent with DNA or siRNA, and then add the transfection complex directly into the cultured cells. Serum does not affect the transfection effect, and there is no need to deliberately add or replace the culture medium.

Protocol:

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

A. Cell seeding:

1. One day before transfection, 0.8-1.5×10^5 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 Lip2000SSS Prime/DNA complex (transfection should be carried out immediately after this step):

1. Add 40μl Trans buffer S into a 1.5 ml sterile centrifuge tube, then add 1μl Lipo5000 Prime, mix gently with a pipette. For other specifications of culture plates, see the attached table 1.
2. Add 2ul of Buffer A to the above centrifuge tube, mix gently, then add 0.8μg DNA, mix gently again with a pipette. Incubate the Lip2000SSS Prime/DNA complex at room temperature for 15 minutes. For other specifications of culture plates, see the attached table 1 for the specific amounts of Buffer A and DNA.
   Note: Use polypropylene centrifuge tubes.

C. Transfection:

1. Add the Lip2000SSS Prime/DNA complex prepared in step B to cells. Mix gently by rocking the plate back and forth.
2. Analyze transfected cells after 24h of incubation or later (36 h - 48 h is recommended). If needed, replace the medium with fresh growth medium after 24 hours of incubation, but it is not necessary.
3. Collect cells for subsequent experiments. For stable transfection, passage cells into selective medium 24 - 48 hours post-transfection.
   Note: Lip2000SSS Prime still has a high transfection efficiency in complete growth medium, so there is no need to change the medium in cell culture plate to serum-free or low-serum culture medium before or 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 should be 50% confluent 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 Lip2000SSS Prime/siRNA complex (transfection should be carried out immediately after this step):

1. Add 40μl Trans buffer S into a 1.5 ml sterile centrifuge tube, then add 1μl Lipo5000 Prime, mix gently with a pipette. For other specifications of culture plates, see the attached table 2.
2. Add 36pmol of siRNA to the above centrifuge tube, mix gently with a pipette. Incubate the Lip2000SSS Prime/siRNA complex at room temperature for 15 minutes. For other specifications of culture plates, see the attached table 2 for the specific amounts of siRNA.
   Note: Use polypropylene centrifuge tubes.

C. Transfection:

1. Add the Lip2000SSS Prime/siRNA complex prepared in step B to cells. Mix gently by rocking the plate back and forth.
2. Incubate cells at 37°C for 24-72 hours prior to testing for knockdown of gene expression. If needed, replace the medium with fresh growth medium after 24 hours of incubation, but it is not necessary. If you want to observe Fam-siRNA transfection, please aspirate the culture medium containing fluorescence and wash the cells twice with 0.01M PBS or fresh culture medium to prevent the fluorescence background from being too high and affecting the observation effect.
   Note: Lip2000SSS Prime still has a high transfection efficiency in complete growth medium, so there is no need to change the medium in cell culture plate to serum-free or low-serum culture medium before or 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, Lip2000SSS Prime can be optimized by using 0.8μl, 1.0μl, or 1.2μl; For 24-well plate siRNA transfection, the Lip2000SSS Prime dosage per well is 1μl, siRNA can be optimized by using 24pmol, 36pmol, 48pmol.
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 40-60% 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. If you are transfecting multiple wells, you can prepare the transfection complex in the same tube and distribute it to each well.

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)	10	20	40	80	160	800
	Buffer A(μl)	0.5	1.0	2.0	4.0	8.0	40
	Lip2000SSS Prime (μl)	0.25	0.5	1.0	2.0	4.0	20
	1μg/μl plasmid (μl)	0.2	0.4	0.8	1.6	3.2	16
Complete medium(ml)		0.12	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)	10	20	40	80	160	800
	Lip2000SSS Prime (μl)	0.25	0.5	1.0	2.0	4.0	20
	siRNA (pmol)	9	18	36	72	140	700
Complete medium(ml)		0.12	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.