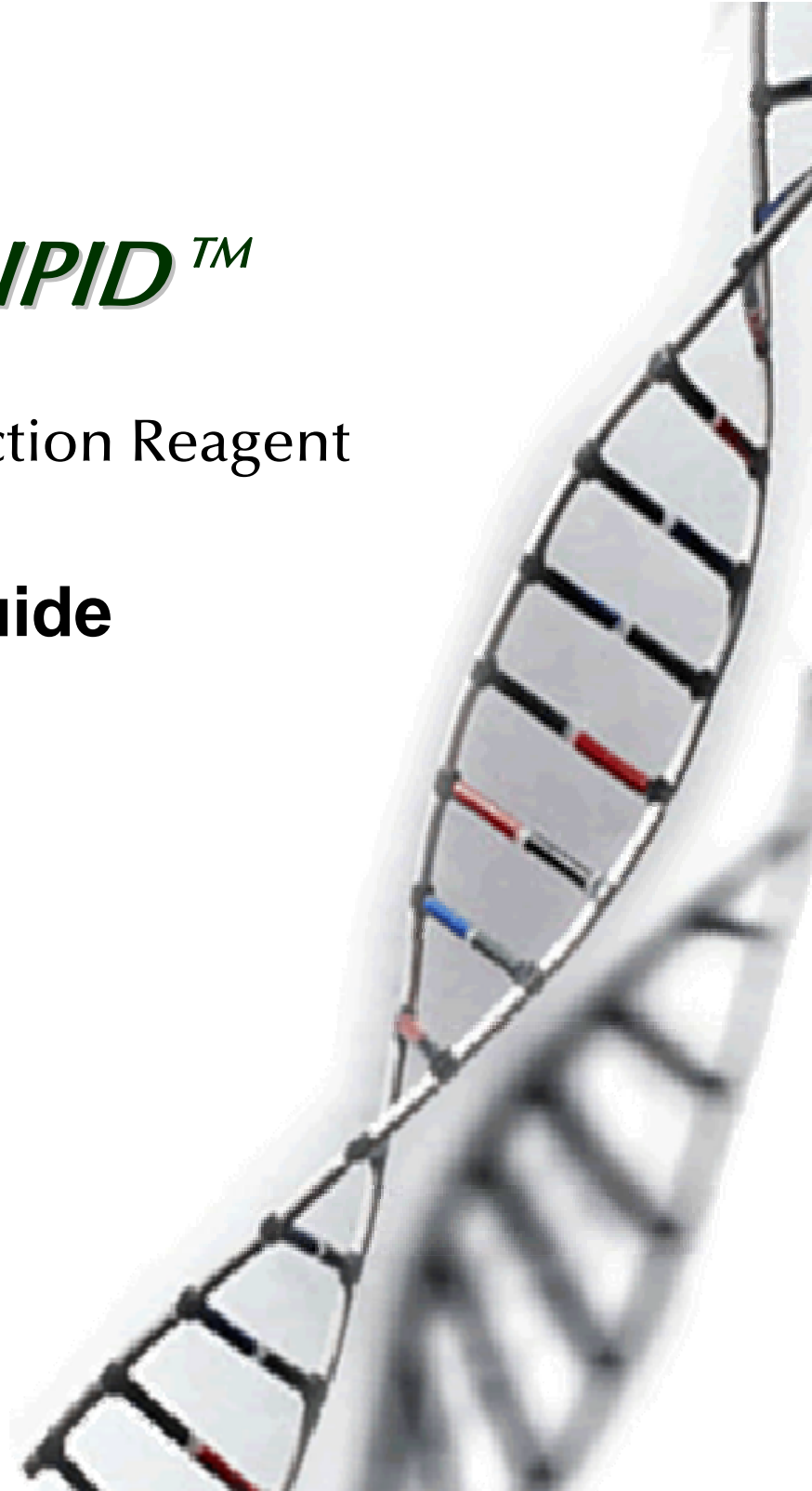


S-LIPID™

OriGene Transfection Reagent

Application Guide



OriGene S-LIPID™ Transfection Reagent – Application Guide

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Package Contents and Storage Conditions

Package Contents:

The S-LIPID™ Transfection Reagent contains proprietary lipids optimized for the delivery of nucleic acids into mammalian cells. The S-LIPID™ Transfection Reagent is available in two formats: 1) as one-hundred microliters per tube (TT100100), and 2) as 0.75 milliliters per tube (TT100200). The former is sufficient for 20 to 30 cell transfections in 35 mm plates and the latter is sufficient for 250 to 300 cell transfections in 35 mm plates with 1 µg DNA per well.

Storage and Stability

The S-LIPID™ Transfection Reagent is **shipped on ice** and should be stored at 4 °C upon receipt. **Do not store below 0 °C.**

Other Required Reagents

- Serum-free media (OptiMEM™) from Invitrogen
- Plasmid DNA (TrueClone cDNA or Hush shRNA)
- Host cell lines (adherent cells or suspension cells)
- Sterile deionized water

Related Products

- TrueClone™ cDNA clones: <http://www.origene.com/cdna/>
- HuSH™ shRNA Plasmids <http://www.origene.com/rna/>
- Validated Antibodies <http://www.origene.com/antibody/>
- Functional Proteins <http://www.origene.com/protein/>

Introduction

Overview

The study of gene function in cultured mammalian cells requires the delivery of specifically designed nucleic acids (recombinant DNAs and short oligos) into the cells. The success of the experiment partly relies on the delivery methods and agents. Two of the most important criteria in measuring this process are transfection efficiency and cellular toxicity. In order to obtain meaningful results, some experiments require over ninety percent of cells to be transfected with targeted nucleic acids, while retaining cell viability.

In the past few decades, several transfection methods have been developed and used effectively for delivering DNA into cultured cells. The delivery methods are classified into chemical and physical approaches. The physical methods include microinjection and electroporation. The chemical methods are most commonly used because they are easy to perform and are cost effective, and include calcium phosphate, DEAE-Dextran, Polybrene and cationic lipids. Cationic lipid based methods are widely used in transfection experiments because of their ability to deliver nucleic acids into a variety of cell lines with high efficiency.

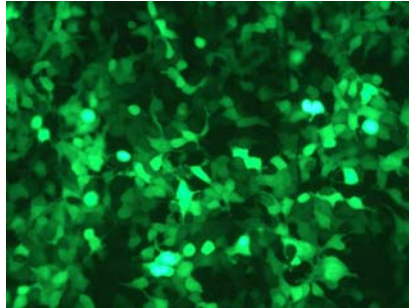
Currently, many cationic lipid based transfection reagents are commercially available and serve the needs of researchers. There remains, however, room for improvement in these types of reagents. This is driven by the introduction of new cell lines in research and new approaches requiring even higher transfection efficiencies. The S-LIPID, cationic lipid based, transfection reagent has been developed to meet these needs. This new product has very high transfection efficiencies and lower cell toxicities in many cell types. S-LIPID is very dependable and is suited for the daily needs of cell biology laboratories involved in cell transfection.

Description of the OriGene S-LIPID™ Transfection Reagent

The S-LIPID™ Transfection Reagent is a mixture of a proprietary cationic lipid compounds and a co-lipid. These compounds have been optimized for the delivery of nucleic acids into cultured mammalian cells in the presence of serum at cell densities up to 70 to 90%. The major advantage of using the S-LIPID™ transfection reagent is its reliability. It can be used to deliver plasmid DNAs and short oligos to a variety of cell lines. For most cell lines, high levels of expression of delivered DNAs can be routinely obtained using the concentrations and ratios of S-LIPID™ and DNAs suggested in the

manual for given sizes of plates /wells. A sample of the results using S-LIPID™ is shown in Fig. 1. For some cell lines, the best transfection conditions may be determined by experimenting with the concentration and ratios of S-LIPID™ and plasmid DNA.

Figure 1. HEK-293 cell transfected with plasmid DNAs expressing green fluorescence protein (GFP) using the S-LIPID™ transfection reagent



How do I use the S-LIPID™ Transfection Reagent?

- Remove the S-LIPID™ Transfection Reagent from the storage refrigerator and mix the tube before use.
- In general, use a ratio of DNA (μg) to lipid (μl) of 0.5:1 to 1:3 to achieve satisfactory transfection results.
- The S-LIPID™ Transfection Reagent is suitable for serum medium, but can be used under serum-free or reduced medium.
- Use sterile polystyrene plastic-ware to prepare the plasmid DNA and lipid mixture. Polypropylene cannot be used because cation-plasmid complexes may bind to it.
- Use cultured cells growing in log phase.

Production and Quality Assurance

Each lot of the S-LIPID™ transfection reagent is tested to have high efficiency for the transfection of plasmid DNA carrying a reporter gene and to be free of microbes.

Methods

Protocol for Transfection of Adherent Cells (24 Well Plate)

1. The day before transfection, inoculate 24 well plates with an appropriate number of cells in a serum containing medium such that the cells will be at 70 to 90% confluence on the following day. Incubate the cells at 37 °C in a CO₂ incubator overnight.
2. Prepare the nucleic acid in 50 μl of serum-free media (OptiMEM from Invitrogen is preferred). Use 0.25 to 1.0 μg of plasmid DNA for each 50 μl of the media.
3. Prepare the S-LIPID™ transfection reagent in 50 μl of serum-free media. Use 0.25 to 4.0 μl as a starting point for optimizing the reaction. To set up the test, use the following 4 compositions listed in Table II. These ratios usually give a range of degree of expression: from none to maximal expression with minimal toxicity.

Table II. Suggested amount of DNA and S-LIPID™ for optimizing the transfection conditions.

Number	1	2	3	4
OptiMEM (µl)	50	50	50	50
S-LIPID™ (µl)	0.25	0.5	1.0	2.0
DNA (ng)	250	500	1,000	4,000

4. Gently mix the DNA and lipid solutions together by tapping or pipetting. Incubate the solution for 20 minutes at room temperature.
5. Add 100 µl of the DNA/Lipid complex directly to cells in serum containing medium. Swirl the plate gently. Incubate the cells in a CO₂ incubator.
6. For transient expression, assay for the anticipated reporter gene activity 24 to 48 hours after the transfection.
7. For stable transfection, remove the medium 24 hours after the transfection, and trypsinize the cells. Transfer the cells to a fresh plate with growth media containing no selective agent. On the following day, replace the media with new media containing the selective agent to be employed. Incubate the plate for 1 to 2 weeks to allow growth of the cells expressing the anti-selective agent.

Protocol for Transfection of Suspension Cells (24 Well Plate)

1. The day before transfection, dilute the cells at 1:3 or 1:4 and place the cells at 37 °C in a CO₂ incubator overnight so that they are in good condition on the day of transfection.
2. Prepare plasmid DNA in 50 µl of serum-free media. Use 0.25 to 1.0 µg of plasmid DNA for each 50 µl medium.
3. Prepare the S-LIPID™ transfection reagent in 50 µl of serum-free media. Use 0.25 to 4.0 µl of lipid for each 50 µl medium.
4. Gently mix the DNA/lipid solutions together by tapping or pipetting. Incubate the mixture for 20 minutes at room temperature.
5. Add 100 µl of DNA/lipid solution to the cell suspension; rock the plates gently.
6. Incubate the cells at 37 °C in a CO₂ incubator. Additional medium may be added 4 to 6 hours after addition of the complexes.
7. For transient transfection, assay the reporter gene activity 24 to 48 hours after transfection.

Trouble-Shooting Guide

For further information, please call our technical support specialists at (888) 267-4436 (US only) or (301) 340-3188.

Frequently Asked Questions

Has S-Lipid been validated in a broad range of cell lines?

Answer: S-Lipid has been shown to be highly efficient in the following cell lines:

HEK-293
CHO
COS
HeLa

3T3
A549
K562
BE(2)C
Primary Fibroblast
Jurkat
BHK

Does S-Lipid display low toxicity?

Answer: In a trypan blue study, S-Lipid displays lower toxicity than the market leading product

DNA : Transfection Reagent	Dead Cells / Total Cells	Ratio
S-Lipid: 1:3	35/158	22.20%
S-Lipid: 1:9	84/143	58.70%
Competitor Lipid : 1:3	37/229	16.20%
Competitor Lipid: 1:9	113/159	71.10%

Is it possible to co-transfect and if so, is there an optimal ratio of nucleic acids?

Answer: Yes, please refer to Table II above

Does OriGene provide cell line-specific protocols?

Answer: OriGene has used S-Lipid in a number of internal assays and it has been used in a broad range of cell lines (see above) and we feel each customer can successfully use the general protocol described in this Guide.

Can I scale up my transfection and if so, what is the optimal ratio?

Answer: The total amount of the DNA complex will increase proportionately to the cell surface (adherent cells) or media volume (suspension cells).

For Jurkat (suspension) cells, can I use PHA and PMA?

Answer: Yes.

If I used polypropylene tubes, should I continue the experiment or start over?

Answer: OriGene recommends starting with fresh complex to have optimal results.