

siTranTM siRNA Transfection

Application Guide

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Package Contents

The following components are included:

- siRNA Transfection Reagent siTran 1.0, (0.5 mL, 1 mL or 5x1 mL)
- One Application Guide

Storage conditions

siTran 1.0 should be stored at 4°C and is stable for at least 6 months under proper storage conditions.

Related OriGene Products

TrueClone™ FL cDNA clones	http://www.origene.com/cdna
Trilencer-27 siRNA	http://www.origene.com/siRNA
HuSH™ shRNA Plasmids	http://www.origene.com/rnai
VERIFY™ Tagged Antigens	http://www.origene.com/lysate/
Validated Antibodies	http://www.origene.com/antibody
Functional Proteins	http://www.origene.com/protein
4C5-AntiDDK Antibody	http://www.origene.com/antibody/4C5-AntiDDK
2H8-Antit-GFP Antibody	http://www.origene.com/antibody/2H8-AntitGFP

Notice to purchaser

This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The plasmids may not be distributed, resold, modified for resale or used to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund.

Introduction

Introducing siRNA duplexes into cells efficiently requires the involvement of a transfection reagent. OriGene has developed a proprietary cationic lipid based transfection reagent, siTran 1.0, for this purpose. siTran 1.0 transfects siRNA into mammalian cells with a high efficiency and has no reported negative effects on cell viability (Fig. 1 A, 1B). siTran 1.0 also displays high efficiency for transfecting plasmid DNA into cells (Fig. 2A). This dual functionality allows one to easily conduct experiments requiring co-transfection of siRNA and plasmid DNA.

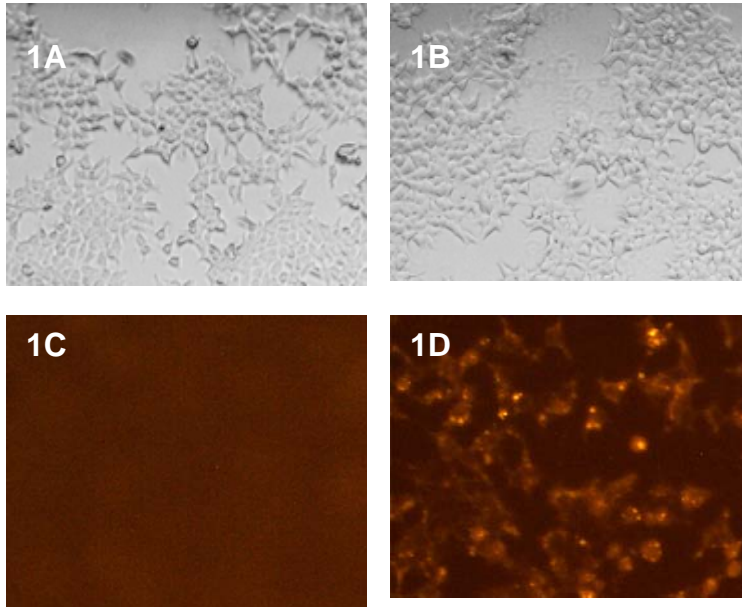


Figure 1. 70% confluent HEK293 cells in a 96-well microtiter plate were transfected with TYE 563 labeled siRNA. Phase contrast (1A, 1B) and fluorescence (1C, 1D) images for the wells with (1A and 1C) or without (1B and 1D) the additions of siTran 1.0 were taken 24 hrs post-transfection.

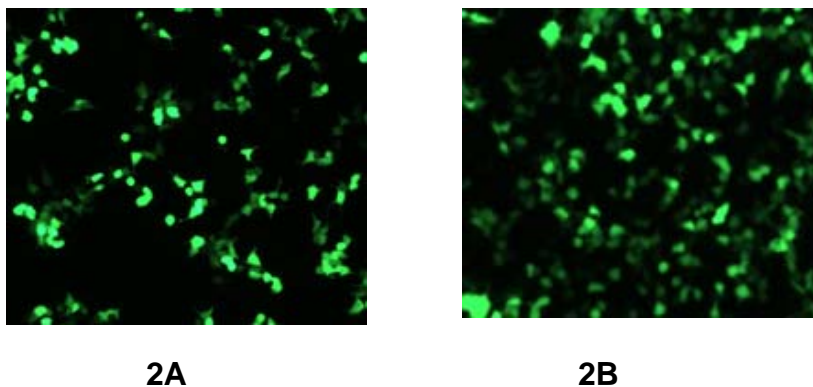


Figure 2. 70% confluent HEK293 cells in a 96-well microtiter plate were transfected with tGFP plasmid DNA using siTran 1.0 (2A) or Turbofectin 8.0 (2B). Fluorescence images were taken 48 hrs post-transfection.

Experimental Procedures

Transfection Optimization

Although the transfection protocol below has been shown to result in highly efficient transfection, we recommend that the transfection conditions be optimized for each distinct cell line. The following variables should be considered:

- A. Cell density (% confluence at transfection): The recommended confluency for most adherent cell types at transfection is 50-70%. The optimal confluency may vary significantly among different cell lines.
- B. siTran 1.0 to siRNA ratio: We suggest that the conditions should be optimized for each cell line using the conditions in table 1 as a starting point.

Table 1. Recommended starting transfection conditions for siRNA using siTran 1.0

Plate Type	final Vol. (ul)	Vol. siRNA (5.0 uM), (10 nM final)	SiTran1.0	Opti-MEM
96 well	120	0.3 ul	1.5 ul	8.5 ul
48 well	340	0.6 ul	3.0 ul	17 ul
24 well	680	1.20 ul	6.0 ul	34 ul
12 well	1200	2.0 ul	10.0 ul	60 ul
6 well	2800	4.0 ul	20.0 ul	120 ul

Table 2. Recommended starting transfection conditions for siRNA and plasmid DNA in a co-transfection using siTran 1.0

Plate Type	final Vol. (ul)	Vol. siRNA (5.0 uM), (10 nM final)	Plasmid DNA	siTran	Opti-MEM
96 well	120	0.3 ul	30 ng	1.5 ul	8.5 ul
48 well	340	0.6 ul	60 ng	3.0 ul	17 ul
24 well	680	1.20 ul	120 ng	6.0 ul	34 ul
12 well	1200	2.0 ul	180 ng	10.0 ul	60 ul
6 well	2800	4.0 ul	360 ng	20.0 ul	120 ul

Protocol for transient transfection in a 96 well plate

1. Plate cells the day before the transfection. Plate the appropriate dilution of cells to provide a confluency of 50-70% in 24 hrs.
2. Dilute siRNA to 5 uM using sterilized duplex buffer supplied by siRNA manufacturer.
3. Set up a series of sterilized 0.2 ml PCR tubes. Add 10 ul of Opti-MEM and 0.3 ul of 5 uM siRNA to each well and also 50 ng plasmid DNA if performing cotransfection.
4. Prepare adequate diluted siTran 1.0 (10 ul per well) in Opti-MEM using the recommended ratio siTran (1.5ul siTran/8.5 ul Opti-MEM) and/or ratios suitable for your cell line. Add 10 ul of the siTran dilution to each PCR tube with siRNA solution.
5. Mix the solution and incubate at room temperature for 10 mins.
6. Transfer the siTran/siRNA mixtures to each well of cells in the microtiter plate
7. Incubate the plate at 37°C in a CO2 incubator.
8. View cells under a fluorescence microscope or harvest cells 24 to 48 hrs after transfection.

Troubleshooting Guide

Low transfection efficiency

Possible cause: Inefficient complex formation.

Recommended solution: Always vortex the mixture immediately after the addition of the reagent to DNA.

Possible cause: Suboptimal reagent/siRNA ratio.

Recommended solution: Optimize the quantity of transfection reagent added to the fixed amount of siRNA

Possible cause: Poor polyplex/cell surface contact.

Recommended solution: Gently centrifuge the culture plates.

Possible cause: Suboptimal cell confluency.

Recommended solution: Optimize cell-plating conditions. Ensure that adhered cells are 50-70% confluent at the time of transfection. Ensure that suspension cells are in logarithmic growth phase at the time of transfection.

Possible cause: Mycoplasma contamination.

Recommended solution: Mycoplasma infection in cell culture often results in poor and/or nonreproducible transfection. Regularly check your cells for mycoplasma infection.

High cellular toxicity

Possible cause: Toxic siRNA

Recommended solution: Verify if the siRNA is toxic.

Possible cause: Suboptimal incubation conditions.

Recommended solution: Reduce incubation time of the polyplexes with the cells. Replace the transfection mixture 3-6 hours later with fresh growth medium.

Possible cause: Cell density is too low.

Recommended solution: Increase the plating density of cells used for transfection.