

siTran™ siRNA Transfection

Application Guide

Package Contents and Storage Conditions.....	1
Notice to purchaser	1
Introduction	1
Experimental Procedures	2
Important Guidelines for Transfection:.....	2
Preparation of Working Solution of siTran 2.0 Transfection Buffer (1x):.....	2
Standard siRNA Transfection Protocol	2
Cell Seeding:.....	2
siRNA Transfection:	3
Standard siRNA/DNA Co-transfection Protocol	4
Cell Seeding:.....	4
siRNA/DNA co-transfection:	4
Troubleshooting Guide	5

Package Contents and Storage Conditions

SKU	Components	Storage Condition	Shipping Condition
TT320001	<ul style="list-style-type: none">• 1 vial of siTran 2.0 reagent, 0.5 mL• 1 vial of 5x transfection buffer, 4 mL	siTran 2.0: 4°C 5x transfection butter: RT	Room temperature
TT320002	<ul style="list-style-type: none">• 1 vial of siTran 2.0 reagent, 1 mL• 1 vial of 5x transfection buffer, 8 mL	siTran 2.0: 4°C 5x transfection butter: RT	Room temperature
TT320002P5	<ul style="list-style-type: none">• 5 vials of siTran 2.0 reagent, 1 mL• 5 vial of 5x transfection buffer, 8 mL	siTran 2.0: 4°C 5x transfection butter: RT	Room temperature

Related OriGene Products

[Trilencer 27-mer siRNA kit](#) – Guaranteed knockdown

[Expression cDNA clones/vectors](#) - Tagged and untagged, ready for transfection

[shRNA plasmids](#)- Human, Mouse and Rat

[CRISPR vectors, gene knockout kits](#)

[qPCR primer pairs](#)

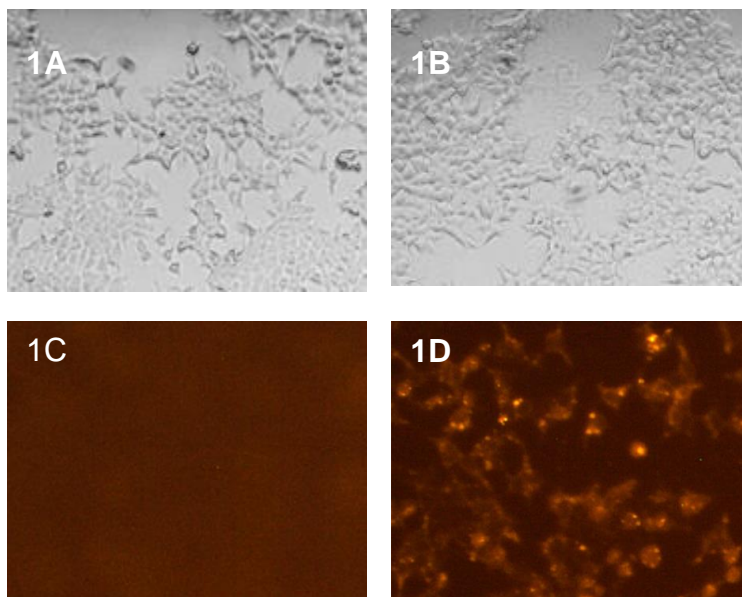
[Primary antibodies](#)

Notice to purchaser

This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited.

Introduction

siRNA is a simple and efficient gene knockdown tool. To achieve gene knockdown, siRNA duplexes need to be delivered into cells by a transfection reagent. OriGene has developed a proprietary polymer-based transfection reagent, siTran 2.0, for this purpose. With our proprietary pH Dependent Conformational Change technology, the polymer was chemically modified by addition of pre-screened hydrophobic groups to the side chain, making siTran 2.0 Reagent a versatile and most powerful gene delivery tool. SiTran 2.0 Reagent have been validated to effectively and reproducibly transfect siRNA, DNA or co-transfect DNA/siRNA to a variety of mammalian cells (Fig. 1 and 2).



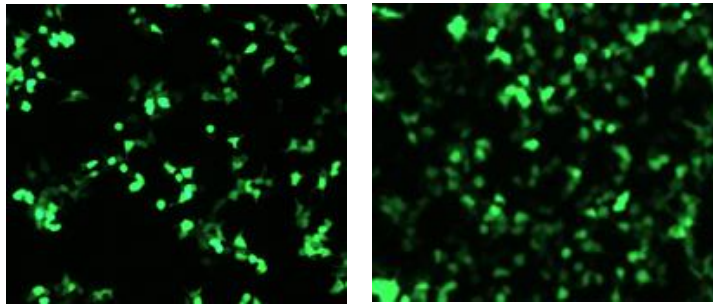


Figure 1. 70% confluent HEK293 cells in a 96-well microtiter plate were transfected with Cy3 labeled siRNA. Phase contrast (1A, 1B) and fluorescence (1C, 1D) images for the wells without (1A and 1C) or with (1B and 1D) the additions of siTran 1.0 were taken 24 hrs post-transfection.

Figure 2. 70% confluent HEK293 cells in a 96-well microtiter plate were transfected with tGFP plasmid DNA using siTran 2.0 (2A) or Turbofectin 8.0 (2B). Fluorescence images were taken 48 hrs post-transfection.

Experimental Procedures

Important Guidelines for Transfection:

1. For maximum gene silencing, please use 1x siTran 2.0 Transfection Buffer working solution to dilute siRNA/DNA and siTran 2.0 Reagent is a must.
2. While the standard protocols for siRNA transfection and siRNA/DNA co- transfection are being given below, optimization is often needed for maximal gene silencing.

Preparation of Working Solution of siTran 2.0 Transfection Buffer (1x):

siTran 2.0 Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make the working solution (1x), dilute one part of the stock solution with 4 parts of dH₂O into a sterile tube/bottle. The working solution is stable at room temperature for 24 months.

Note: Always keep siTran 2.0 Transfection Buffer working solution (1x) at room temperature. Always keep the Transfection Buffer (5x) at room temperature. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After diluting with 4 parts of dH₂O to make the 1x working solution, the white precipitates will disappear.

Standard siRNA Transfection Protocol

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 media with serum and antibiotics is freshly added to each well 30~60 min before transfection.

Note: SiTran 2.0 reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing media can be used during the entire experiment.

siRNA Transfection:

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-transfect cells, we recommend using a final siRNA concentration of 50 nM. (bold & underlined in **Table 1**). To optimize transfection efficiency, fluorescent labeled siRNA can be used (cat# [SR30002](#))

The following protocol is given for 6-well plate. For other culture formats, please refer to **Table 1**.

1. Change fresh media: Replace with 1.0 mL of fresh complete medium with serum and antibiotics 30~60 min before transfection.
2. Dilute siRNA in 1x Transfection Buffer:
 - 1) Make 5 uM siRNA stock solution using sterilized duplex buffer supplied by siRNA manufacturer.
 - 2) In a sterile 0.2 mL PCR tube or Eppendorf tube, add 100 uL 1x Transfection buffer, then add 1.1 uL of 5uM siRNA solution, mix by pipetting up and down.
3. Add 2.4 uL siTran 2.0 reagent to the diluted siRNA, mix by pipetting up and down. Incubate for ~15 min at room temperature.
Note: Never keep the complex longer than 30 min.
4. Add the mixture prepared in step 3 drop-wise onto cells. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly. Incubate cells at 37°C.
5. 12~18 hours post transfection, remove transfection complex-containing media and replace with fresh complete serum/antibiotics containing media. **For sensitive cells, to lower the cytotoxicity, change the media 5 hours after adding the transfection complex.**
6. View cells under a fluorescence microscope or harvest cells 24 to 48 hrs after transfection. Measure gene knockdown.

Table 1. A Guideline for siRNA transfection per cell culture vessel

Plate Type	Growth Media (mL)	siRNA, uL of 5 uM stock Final 5.0 or <u>50</u> nM	siTran2.0 (uL)	Transfection Buffer (uL)
24-well	0.5	0.55 / <u>5.5</u>	1.2	50
12-well	0.75	0.82 / <u>8.2</u>	2.0	75
6-well	1.0	1.1 / <u>11</u>	2.4	100
60 mm	3.0	3.3 / <u>33</u>	7.2	300
10 cm/T75	8.0	8.8 / <u>88</u>	20	800

Standard siRNA/DNA Co-transfection Protocol

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 media with serum and antibiotics is freshly added to each well 30~60 min before transfection.

Note: SiTran 2.0 reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing media can be used during the entire experiment.

siRNA/DNA co-transfection:

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. To optimize transfection efficiency, fluorescent labeled siRNA (cat# [SR30002](#)), GFP expression plasmid (pCMV6-AC-GFP, Cat# [PS100010](#)) can be used.

The following conditions are given per well of a 6-well plate. For other culture format, please refer to **Table 2**.

1. Change fresh media: Replace with 1.0 mL of fresh complete medium with serum and antibiotics 30~60 min before transfection.
2. Dilute siRNA /DNA in 1x Transfection Buffer:
 - 1) Make 5 uM siRNA stock solution using sterilized duplex buffer supplied by siRNA manufacturer.
 - 2) In a sterile 0.2 mL PCR tube or Eppendorf tube, add 100 uL 1x Transfection buffer, then add 2.2 uL of 5uM siRNA solution and 0.5 ug of DNA, mix by pipetting up and down.
3. Add 3 uL siTran 2.0 reagent to the diluted siRNA, mix by pipetting up and down. Incubate for ~15 min at room temperature.
Note: Never keep the complex longer than 30 min.
4. Add the mixture prepared in step 3 drop-wise onto cells. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly. Incubate cells at 37°C.
5. 12~18 hours post transfection, remove transfection complex-containing media and replace with fresh complete serum/antibiotics containing media. **For sensitive cells, to lower the cytotoxicity, change the media 5 hours after adding the transfection complex.**
6. View cells under a fluorescence microscope or harvest cells 24 to 48 hrs after transfection. Measure gene knockdown.

Table 2. A Guideline for siRNA transfection per cell culture vessel

Plate Type	Growth Media (mL)	siRNA, uL of 5 uM stock Final 10 nM	siTran2.0 (uL)	Plasmid DNA (ug)	Transfection Buffer (uL)
24-well	0.5	1.1	1.5	0.25	50
12-well	0.75	1.6	2.25	0.375	75
6-well	1.0	2.2	3	0.5	100
60 mm	3.0	6.6	9	1.5	300
10 cm/T75	8.0	17.6	24	4.0	800

Troubleshooting Guide

Low transfection efficiency

Possible cause: Suboptimal reagent/siRNA ratio.

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

Possible cause: Suboptimal cell confluency.

Recommended solution: Optimize cell plating conditions. Ensure that adhered cells are 50-70% confluent 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 transfection complexes with cells. Replace the transfection mixture 5 hours later with fresh growth medium.

Possible cause: Cell density is too low.

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