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# siPORT™ *NeoFX*™ Transfection Agent

Lipid-based Agent for Reverse Transfection

**Publication Part Number** 4510M Rev. D **Revision Date** September 2011



## Product description

siPORT™ *NeoFX*™ Transfection Agent is a lipid-based formulation that provides extremely high levels of transfection without inducing cellular stress that can lead to cytotoxicity. It can be used with a wide range of cell types† and under diverse growth conditions. The transfection agent is stable over a broad temperature range (–20°C to 22°C), reducing shipping and storage issues common with other popular transfection agents. These key features lead to unsurpassed consistency in well-to-well and day-to-day performance. The fast, easy-to-use transfection procedure ensures minimal hands-on time and high reproducibility in all standard and high throughput siRNA formats.

## Background

The importance of small RNAs such as siRNAs and miRNAs in gene regulation is becoming more and more evident. To overcome the problems with well-to-well variability in delivering small RNAs to cells in culture plates, we developed siPORT™ *NeoFX*™ Transfection Agent and a simpler, more streamlined transfection methodology called reverse transfection.

In this user guide we provide instructions for using siPORT™ *NeoFX*™ Transfection Agent in standard cell culture plates and for high throughput screening of small RNAs such as *Silencer*™ Select siRNAs, *Silencer*™ siRNAs, Pre-miR™ miRNA Precursors, and Anti-miR™ miRNA Inhibitors. We recommend optimizing the transfection procedure for each cell type that will be transfected. Detailed instructions for optimization experiments are included in the *Silencer*® *siRNA Transfection II Kit User Guide* (Part no. 1631M).

† For example, SKOv3, MCF-7, SKNAS, NIH3T3, COS-7, HeLa, A549, BJ, UMR108, HT-29, HepG2

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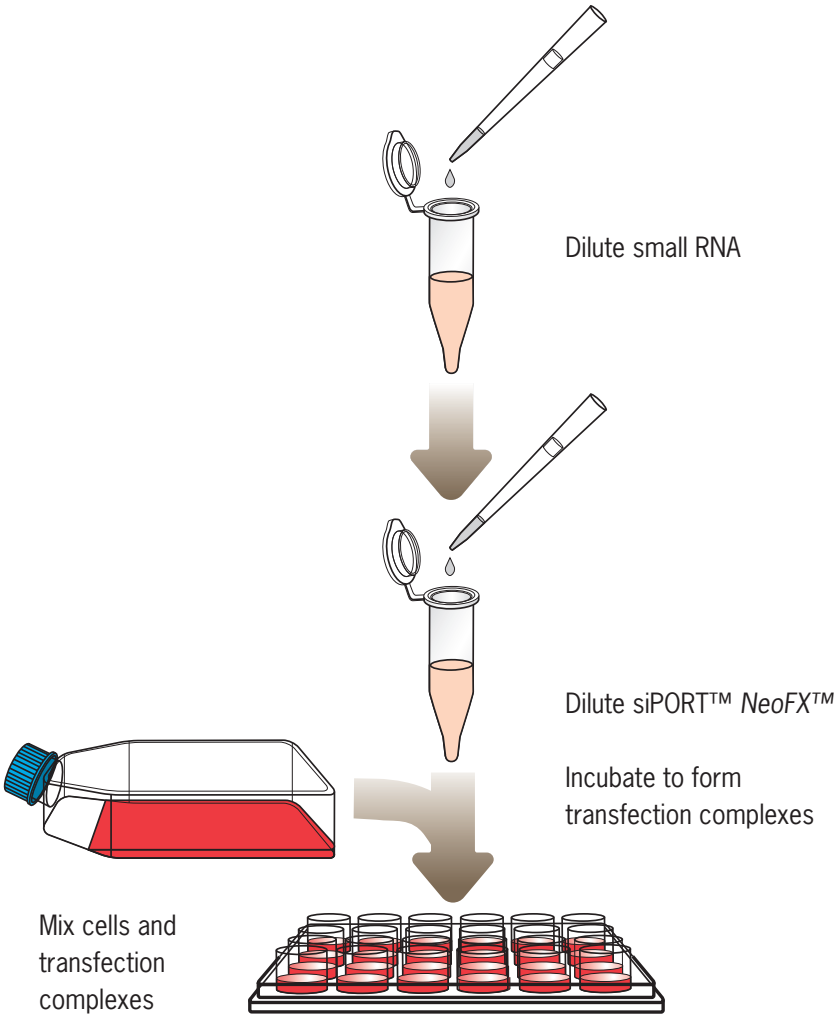
# Reverse transfection overview

Reverse transfection involves simultaneously transfecting and plating cells, much like procedures used for transfecting suspension cells. siPORT™ *NeoFX*™ Transfection Agent and RNA are mixed, incubated, distributed to culture wells, and overlayed with cells. The transfection complexes are active and stable in the presence of serum; therefore, in the absence of cellular stress response, there is no need to remove or replace media after transfection.

**Figure 1** Reverse Transfection Overview



# Transfection procedure

For the most rapid assay development, we provide suggested initial cell and reagent amounts. Use these conditions in your first experiments and optimize as needed based on the results.

- These instructions give examples of reagent amounts to use per well for 96-, 24-, 12-, or 6-well plates. Use the plate format that best suits the needs of your experiment.
- For preparation of RNA and reagent dilutions, use a sterile culture plate (round or V-bottom) or sterile tubes.
- When possible, prepare master mixes to minimize variability.

1. Trypsinize adherent cells and dilute in normal growth medium

   One (1) hour or less before transfection, trypsinize healthy, growing, adherent cells using your routine procedure. Inactivate trypsin by resuspending the cells in normal growth medium (e.g., DMEM, 10% FBS). Set cells aside at 37°C (water bath or incubator) while you prepare the transfection complexes.

   **Note:** Cells will be transfected in suspension shortly after trypsinization. It is important to prepare cell suspensions either before assembling transfection complexes or at the same time because the incubation times for complex formation must be precise for the best results.

2. Dilute siPORT™ NeoFX™ Transfection Agent in Opti-MEM® I medium and incubate 10 min at room temp

   a. Warm siPORT™ *NeoFX*™ Transfection Agent and Opti-MEM® I medium to room temp, and centrifuge siPORT™ *NeoFX*™ Transfection Agent briefly before use.

   b. Dilute siPORT™ *NeoFX*™ Transfection Agent into Opti-MEM® I medium (see the table below for suggested amounts).

| | 96-well | 24-well | 12-well | 6-well |
|---|---|---|---|---|
| siPORT™ NeoFX™ Transfection Agent | 0.5 µL | 1 µL | 3 µL | 5 µL |
| Opti-MEM® medium to: | 10 µL | 25 µL | 50 µL | 100 µL |

**IMPORTANT!** Tightly close siPORT™ *NeoFX*™ Transfection Agent after use to prevent evaporation.

   c. Incubate for 10 min at room temperature.

3. Dilute RNA in Opti-MEM® I medium

   Next, dilute your small RNA into Opti-MEM® I medium. The recommended final RNA concentration (that is, the final concentration after the transfection complexes are mixed with the cells in step 5) is 5 nM for *Silencer*® Select siRNAs and 30 nM for *Silencer*® siRNAs, Anti-miR™ miRNA Inhibitors, and Pre-miR™ miRNA Precursors.

| | 96-well | 24-well | 12-well | 6-well |
|---|---|---|---|---|
| Opti-MEM® to: | 10 µL | 25 µL | 50 µL | 100 µL |
| Add the following amounts of different types of small RNA: | | | | |
| 1 µM *Silencer*® Select siRNA | 0.5 µL | 2.5 µL | 5 µL | 12.5 µL |
| 10 µM other small RNA† | 0.3 µL | 1.5 µL | 3 µL | 7.5 µL |

† *Silencer*® siRNA, Anti-miR™ Inhibitor, or Pre-miR™ Precursor.

4. Mix diluted RNA and diluted siPORT™ *NeoFX*™ Transfection Agent; incubate 10 min at room temp, and dispense into a culture plate

   a. Combine diluted siPORT™ *NeoFX*™ Transfection Agent and diluted RNA. Mix by pipetting up and down or flicking the tube a few times.

   b. Incubate for 10 min at room temp to allow transfection complexes to form.

   c. Dispense RNA/siPORT™ *NeoFX*™ Transfection Agent transfection complexes into wells of a clean culture plate.

5. Overlay cell suspensions onto the transfection complexes and gently tilt the plate to mix.

   a. Gently mix the cells prepared in step 1 to resuspend any that have settled, and pipet them into the culture plate wells containing transfection complexes.

| | 96-well | 24-well | 12-well | 6-well |
|---|---|---|---|---|
| Cell overlay volume | 80 µL | 450 µL | 900 µL | 2.3 mL |
| Total number of cells | $6 \times 10^3$ | $4 \times 10^4$ | $8 \times 10^4$ | $2 \times 10^5$ |
| Final transfection volume | 100 µL | 500 µL | 1 mL | 2.5 mL |

   b. Without swirling, gently tilt the plate back and forth to evenly distribute the complexes.

6. Incubate the transfected cells at 37°C in normal cell culture conditions until ready to assay.

7. Assay for effects of the transfected RNA 8–72 hours after transfection.

The best time to assay will depend on the type of assay being performed and on the gene target. For most applications, assay for target-gene activity 8–72 hr after transfection.

**IMPORTANT!** If transfection causes cytotoxicity, replace the medium with fresh growth medium after 8–24 hr in subsequent experiments. Replacing medium too soon, (e.g. after 4 hr) may result in inefficient transfection and suboptimal effect from the transfected nucleic acid.

# Example High Throughput Transfection Procedure

To limit the amount of hands-on time required for large scale experiments, we developed a high throughput procedure that is both rapid and simple. This method is routinely used at Ambion to transfect collections of small RNAs in high throughput format; it consists of three steps:

- Distribute small RNA to empty wells of a 96-well plate.
- Overlay RNA with diluted siPORT™ *NeoFX*™ Transfection Agent.
- Overlay the small RNA/siPORT™ *NeoFX*™ mixture with cells.

1. Distribute RNA into wells of a 96-well plate.

   For *Silencer*® Select siRNAs, prepare a 1 µM stock solution and dispense 0.5 µL into empty wells of a sterile 96-well culture plate. This will result in a final siRNA concentration of 5 nM.

   For *Silencer*® siRNAs, Pre-miR™ miRNA Precursors, and Anti-miR™ miRNA Inhibitors, prepare a 10 µM stock solution and dispense 0.3 µL into empty wells of a sterile 96-well culture plate. This will result in a final small RNA concentration of 30 nM.

   Use the plate immediately or store at –20°C or –80°C. Alternatively, the contents of the plate can be lyophilized and stored at 4°C or –20°C.

2. Trypsinize adherent cells and dilute in normal growth medium to $10^5$ cells per mL.

   1 hr or less before transfection, trypsinize healthy, growing, adherent cells using your routine procedure. Inactivate trypsin by resuspending the cells in normal growth medium to reach a concentration of $10^5$ cells/mL. Set cells aside at 37°C (water bath or incubator) while you prepare the transfection complexes.

   **IMPORTANT!** Cells will be transfected shortly after trypsinization. It is important to prepare cell suspensions either before assembling transfection complexes or at the same time because the incubation times for complex formation must be precise for the best results.

3. Dilute siPORT™ *NeoFX*™ Transfection Agent in Opti-MEM® I medium and incubate 10 min at room temp.

   a. If plate(s) containing small RNAs was frozen, thaw to room temp. Also, bring the siPORT™ *NeoFX*™ Transfection Agent and the Opti-MEM® I medium to room temp, and centrifuge briefly before use.

b. In a sterile tube, prepare a master mix by diluting siPORT™ *NeoFX*™ Transfection Agent (for example 0.3 µL/well or 30 µL/plate) into Opti-MEM® I medium. Use a final volume of 20 µL per well (2 mL/plate) for RNA in solution, or 30 µL per well (3 mL/plate) for lyophilized RNA.

c. Incubate 10 min at room temp.

4. Add diluted siPORT™ *NeoFX*™ Transfection Agent to the RNA in the plate, mix, and incubate 10 min at room temp.

   a. Pipet the diluted siPORT™ *NeoFX*™ Transfection Agent onto the pre-dispensed small RNA (from step 1 above). Use 20 µL per well diluted siPORT™ *NeoFX*™ Transfection Agent for RNA in solution, or 30 µL per well for lyophilized RNA.

   b. Gently agitate plate to ensure thorough mixing. Incubate at room temp for 10 min.

      If the RNA was lyophilized, then make sure that the transfection mixture completely covers the surface of the wells to thoroughly resuspend the RNA.

5. Overlay cell suspensions and gently tilt the plate to mix.

   Gently mix the cells prepared in step 2 to resuspend any that have settled, and pipet 80 µL of cells per well into the culture plate wells containing transfection complexes. Without swirling, gently tilt the dish back and forth to evenly distribute the complexes.

6. Incubate at 37°C.

   Incubate the transfected cells in normal cell culture conditions until ready to assay.

7. Assay for target gene activity 8–72 hr after transfection.

   If transfection causes cytotoxicity, replace the medium with fresh growth medium after 8–24 hr in subsequent experiments. Replacing medium too soon, (e.g. after 4 hr) may result in inefficient transfection and suboptimal effect from the transfected nucleic acid.

# Transfection optimization overview

Table 1 gives the recommended ranges for optimization of cell number, siPORT™ *NeoFX*™ Transfection Agent volume, and small RNA amount to evaluate in optimization experiments. Detailed instructions for optimization experiments are included in the Ambion *Silencer*® siRNA Transfection II Kit User Guide (Part no. AM1631).

The most important parameter for optimization of small RNA delivery is the amount of siPORT™ *NeoFX*™ Transfection Agent. All other parameters can be considered fine-tuning adjustments. The goal is to establish a balance between knockdown and cytotoxicity. Once conditions are established, keep them constant between experiments for a given cell type.

## Step 1

Test 3 different volumes of siPORT™ *NeoFX*™ Transfection Agent (see Table 1) for knockdown and cytotoxicity. Use 5 nM final concentration of *Silencer*® Select siRNAs or 30 nM final concentration of *Silencer*® siRNA, Anti-miR™miRNA Inhibitor, or Pre-miR™ miRNA Precursor.

**Step 2** If cytotoxicity is encountered, replace media at 8 hr or 24 hr. Re-evaluate knockdown and cytotoxicity.

**Step 3** For optimizing the activity of transfected small RNAs, test different amounts of small RNA (see Table 1) using the siPORT™ *NeoFX*™ Transfection Agent quantity optimized in Step 1.

**Step 4** To optimize overall transfection efficiency, optimize the cell concentration using the transfection conditions identified in the previous steps.

**Table 1** Suggested reagent amounts per well for optimization

| | 96-well | 24-well | 12-well | 6-well |
|---|---|---|---|---|
| Cells per well | 0.5–1 × $10^4$ | 3–6 × $10^4$ | 0.75–1.5 × $10^5$ | 1.5–3 × $10^5$ |
| siPORT™ *NeoFX*™ Transfection Agent | 0.15–0.75 µL | 0.5–2.5 µL | 1–4 µL | 2–8 µL |
| Use the following amounts of different types of small RNA | | | | |
| 1 µM *Silencer*® Select siRNA† | 0.05–2 µL | 0.25–10 µL | 0.5–20 µL | 1.25–50 µL |
| 10 µM other small RNA‡ | 0.1–0.5 µL | 0.5–2.5 µL | 1–5 µL | 2.5–12.5 µL |

† 1 µM *Silencer*® Select siRNA results in a final concentration of 0.5–20 nM.

‡ This results in a final concentration of 10–50 nM. Use this amount of *Silencer*® siRNA, Anti-miR™ Inhibitor, or Pre-miR™ Precursor. We have found that *Silencer*® siRNAs typically work best at 10–50 nM, but a more extensive concentration range from 1–100 nM can be analyzed in optimization experiments.

# Available Related Products

**siPORT™ Amine Transfection Agent (Part no. AM4502, AM4503)**

siPORT Amine is an easy-to-use proprietary blend of polyamines that delivers siRNA into mammalian cells with minimal cytotoxicity.

***Silencer*® Select siRNAs (see web or print catalog for part numbers)**

*Silencer*® Select siRNAs are designed using an all-new algorithm that was developed utilizing the latest in machine learning methods. These next generation siRNAs exhibit up to 100-fold higher silencing potency than siRNAs from other leading siRNA manufacturers. Off-target activity (assayed by microarray analysis) is blocked by up to 90% because *Silencer*® Select siRNAs can be used at 5- to 20-fold lower concentrations, are bioinformatically screened using the latest knowledge about miRNA seed regions and toxic sequence motifs, and incorporate strategic chemical modifications. As a result, *Silencer*® Select siRNAs provide unrivalled specificity and cleaner, more consistent phenotypic data.

***Silencer*® siRNAs (see web or print catalog for part numbers)**

Life Technologies *Silencer*® Pre-designed siRNAs, Validated siRNAs, and siRNA Libraries are designed with the most rigorously tested siRNA design algorithm in the industry. *Silencer*® siRNAs are available for >100,000 human, mouse, and rat targets from our searchable online database. Because of their carefully optimized design,

*Silencer*® siRNAs are very effective, and they are guaranteed to reduce target mRNA levels by 70% or more. Furthermore, their exceptional potency means that *Silencer*® siRNAs effectively induce RNAi at very low concentrations, minimizing off-target effects.

**Pre-miR™ miRNA Precursors (Part no. AM17100, AM17101, AM17103)**

Pre-miR™ miRNA Precursors are small, chemically modified double-stranded RNA molecules designed to mimic endogenous mature miRNA molecules. These ready-to-use miRNA mimics can be introduced into cells using transfection or electroporation parameters similar to those used for siRNAs and enable detailed study of miRNA biological effects via gain-of-function experiments. Pre-miR™ miRNA Precursors are available for all miRNAs listed in the miRBase database and custom design is available.

**Anti-miR™ miRNA Inhibitors (Part no. AM17110, AM17111)**

Anti-miR™ miRNA Inhibitors are chemically modified, single-stranded nucleic acids designed to specifically bind to and inhibit endogenous microRNA (miRNA) molecules. These ready-to-use inhibitors can be introduced into cells using transfection or electroporation parameters similar to those used for siRNAs, and enable miRNA functional analysis by down-regulation of miRNA activity.

***Silencer*® siRNA Libraries (see web or print catalog for part numbers)**

Life Technologies offers siRNA libraries to select gene groups. All of the siRNAs included in the *Silencer*® siRNA Libraries have been designed using a proven algorithm developed by Cenix BioScience. This design algorithm yields a high percentage of active siRNAs. Multiple siRNAs per target are provided for enhanced confidence in gene silencing data.

**KDalert™ GAPDH Assay Kit (Part no. AM1639)**

The KDalert GAPDH Assay Kit is a rapid, convenient, fluorescence-based method for measuring the enzymatic activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in cultured human, mouse, or rat cells. The KDalert GAPDH Assay Kit facilitates identification of optimal siRNA delivery conditions by assessment of GAPDH expression and knockdown at the protein level and integrates seamlessly with the Silencer® CellReady siRNA Transfection Optimization Kit (Part no. AM86050) and *Silencer*® GAPDH Control siRNAs (Part no. AM4605, AM4624).

# Appendix A Safety

**WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

## Biological hazard safety

**WARNING! Potential Biohazard.** Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

## Chemical safety

**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

# Documentation and Support

## Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**

**Note:** For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

## Obtaining support

For the latest services and support information for all locations, go to:

**www.lifetechnologies.com**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches



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