# siPORT™ *Amine* Transfection Agent

**Polyamine-Based Transfection Agent**
**Part Number AM4502, AM4503**

Applied Biosystems

## A. Introduction

siPORT™ *Amine* Polyamine-Based Transfection Agent is a proprietary blend of polyamines formulated for transfection of small RNAs. The reagent functions by complexing with RNA and facilitating its transfer into cells. siPORT *Amine* Transfection Agent is easy to use and has minimal cytotoxic effects.

## B. Background

Successful delivery of small RNA into cells is dependent on several variables and different cell types often respond differently to transfection conditions; it may, therefore, be necessary to optimize transfection conditions for each cell type under study. The procedures provided in this Protocol should be used as a starting point to determine if further optimization is necessary. Section F on page 7 provides guidelines for optimization.

Instructions for two alternative transfection methods are provided: pre-plated and reverse transfection. In pre-plated (or forward) transfection, cells are plated ~24 hr before the experiment. Reverse transfection is a method in which cells are transfected as they adhere to a plate after trypsinization; it bypasses several steps of the traditional pre-plating transfection method, making it faster and easier.

Either transfection methodology can be used with siPORT *Amine* Transfection Agent, but we recommend trying pre-plated transfection first. We have found that siPORT *Amine* Transfection Agent often delivers more consistent transfection results using the pre-plated transfection methodology, but in some cases, reverse transfection works just as well.

## C. Transfection Tips

### Determine if optimization is needed

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 using the suggestions in section F on page 7.

### Suggestions for successful transfection

- 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.

### Choose transfection method

Instructions for pre-plated transfection are given in section D and for reverse transfection in section E. We recommend trying pre-plated transfection first, however, in some cases, both methods may be effective.

## D. Pre-Plated Transfection Procedure

### 1. Pre-plate cells (1 day prior to transfection)

a. Approximately 24 hr before transfection, trypsinize healthy adherent cells using your routine procedure. Inactivate trypsin by resuspending the cells in normal growth medium (e.g., DMEM, 10% FBS).

b. Plate cells in normal growth medium so that they will reach 30–80% confluency after 24 hr (the table below shows approximate cell numbers to plate).

| | 96-well | 24-well | 12-well | 6-well |
|---|---|---|---|---|
| Cell plating volume | 80 µL | 450 µL | 900 µL | 2.3 mL |
| No. of cells plated | $6 \times 10^3$ | $4 \times 10^4$ | $8 \times 10^4$ | $2 \times 10^5$ |
| Final transfection vol. | 100 µL | 500 µL | 1 mL | 2.5 mL |

c. Incubate cells overnight using normal cell culture conditions.

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

a. Bring siPORT *Amine* Transfection Agent and OPTI-MEM I medium (Invitrogen) to room temp before use. Centrifuge siPORT *Amine* agent briefly before use to collect liquid at the bottom of the tube.

b. Dilute siPORT *Amine* agent into OPTI-MEM I medium (see the table below for suggested amounts).

| | 96-well | 24-well | 12-well | 6-well |
|---|---|---|---|---|
| siPORT™ *Amine* agent | 0.3 µL | 1.5 µL | 3 µL | 5 µL |
| OPTI-MEM® to: | 10 µL | 25 µL | 50 µL | 100 µL |

**IMPORTANT**

*Tightly close the container of siPORT Amine Transfection Agent immediately after use to prevent evaporation.*

c. Incubate 10 min at room temp.

**3. Dilute RNA in OPTI-MEM I medium**

Dilute RNA into OPTI-MEM I medium. The recommended final RNA concentration 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 *Amine* agent and incubate for 10 min at room temp**

a. Combine the diluted siPORT *Amine* Transfection Agent from step 2 with the diluted RNA from step 3. Mix by pipetting up and down or flicking the tube.

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

**5. Mix the transfection complexes with cells**

Dispense the nucleic acid/siPORT *Amine* transfection complexes onto the cells. Without swirling, gently tilt the plate 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 effects of transfected RNA**

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

NOTE

*If transfection appears to cause 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 effects from the transfected nucleic acid.*

## E. Reverse Transfection Procedure

**1. Trypsinize adherent cells and dilute in normal growth medium**

a. 1 hr or less before transfection, trypsinize healthy, growing, adherent cells using an established procedure.

b. Inactivate trypsin by resuspending the cells in normal growth medium (e.g., DMEM, 10% FBS). Keep the cells at 37°C while preparing the transfection complexes (below).

**NOTE**

*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 *Amine* Transfection Agent in OPTI-MEM I medium and incubate 10 min at room temp**

a. Warm siPORT *Amine* Transfection Agent and OPTI-MEM I medium (Invitrogen) to room temp, and centrifuge siPORT *Amine* Transfection Agent briefly before use.

b. Dilute siPORT *Amine* agent into OPTI-MEM I medium (see the table below for suggested amounts).

| | 96-well | 24-well | 12-well | 6-well |
|---|---|---|---|---|
| siPORT *Amine* agent | 0.3 µL | 1.5 µL | 3 µL | 5 µL |
| OPTI-MEM I medium to: | 10 µL | 25 µL | 50 µL | 100 µL |

**IMPORTANT**

*Tightly close the siPORT Amine Transfection Agent after use to prevent evaporation.*

c. Incubate for 10 min at room temp.

**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 cells in step 5) is 5 nM for *Silencer* Select siRNAs and30 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 *Amine* Transfection Agent; incubate 10 min at room temp, and dispense into a culture plate**

a. Combine diluted siPORT *Amine* Transfection Agent from step 2 with diluted RNA from step 3. Mix by pipetting up and down or by flicking the tube a few times.

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

c. Dispense RNA/siPORT *Amine* 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 vol. | 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 at 37°C**

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

**7. Assay for effects of the transfected RNA**

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

NOTE

*If transfection appears to cause 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 effects from the transfected nucleic acid.*

## F. Transfection Procedure Optimization

Table 1 gives the recommended ranges for optimization of cell number, siPORT *Amine* 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 Protocol, available for download from our website:

www.ambion.com/techlib/prot/fm_1631.pdf

The most important parameter for optimization of small RNA delivery is the amount of siPORT *Amine* 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 *Amine* Transfection Agent (see Table 1) for knockdown and cytotoxicity. Use 5 nM final concentration of *Silencer* Select siRNA or 30 nM S*ilencer* 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 a larger range of small RNA quantities (following the guidelines in Table 1) using the siPORT *Amine* agent quantity optimized inG 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 Optimization Ranges.

| Variable | Culture Vessel | | | |
|---|---|---|---|---|
| | 96-well | 24-well | 12-well | 6-well |
| Cells per well | 0.5–1 x $10^4$ | 3–6 x $10^4$ | 0.75–1.5 x $10^5$ | 1.5–3 x $10^5$ |
| siPORT™ *Amine* agent | 0.15–0.75 µL | 0.5–2.5 µL | 1–5 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 |

* This 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.

## G. Related Products

### siPORT™ *NeoFX*™ Transfection Agent

P/N AM4510, AM4511

siPORT *NeoFX* Transfection Agent was developed to streamline siRNA transfection procedures, cutting time and increasing reproducibility. This novel lipid-based formulation can be used to efficiently transfect adherent cells while subculturing, without increased cytotoxicity. It is compatible with a wide range of cell lines and experimental designs, including high-throughput applications.

### *Silencer*® Select siRNAs

See web or print catalog for P/Ns

**(www.ambion.com/geneassist)**

*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. Search the GeneAssist™ Atlas at **www.ambion.com/geneassist** to find guaranteed-to-silence siRNAs to your gene of interest.

### *Silencer*® siRNAs

See web or print catalog for P/Ns
(**www.ambion.com/siRNA**)

Ambion *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.

### Ambion® Pre-miR™ miRNA Precursors

P/N 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.

### Ambion® Anti-miR™ miRNA Inhibitors

P/N 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 P/Ns

Ambion 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 Ambion's partner, 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. See our website for more information at: www.ambion.com/siRNA

KDalert™ GAPDH Assay Kit

P/N 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 (P/N AM86050) and *Silencer* GAPDH Control siRNAs (P/N AM4605, AM4624).

## H. Quality Control

**Functional testing**

Cells in culture are transfected using siPORT *Amine* Transfection Agent with a negative control siRNA or an siRNA targeting luciferase gene. The percent knockdown of the luciferase target is then evaluated to ensure that each lot of siPORT *Amine* Transfection Agent can efficiently deliver siRNAs.

**RNase activity testing**

siPORT *Amine* Transfection Agent is tested for RNase using the Ambion RNaseAlert® assay.

## I. Safey Information

**Chemical safety guidelines**

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety goggles, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

### About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

## J. Material Safety Data Sheets

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At **www.appliedbiosystems.com**, select **Support**, then **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At **www.ambion.com**, go to the web catalog page for the product of interest. Click **MSDS**, then right-click to print or download.
- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com), telephone (650-554-2756; USA), or fax (650-554-2252; USA) your request, specifying the catalog or part number(s) and the name of the product(s). The associated MSDSs will be e-mailed unless you request fax or postal delivery. Requests for postal delivery require 1 to 2 weeks for processing.

For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

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