siPORT™ *XP-1* Transfection Agent

Part Number AM4507

A. Product Description

siPORT™ *XP-1* is an easy-to-use, proprietary formulation of polyamines for transfecting DNA into eukaryotic cells with high efficiency and low cytotoxicity. It is suitable for the transfection of a wide variety of cultured mammalian cell types with both plasmid DNA and PCR products. siPORT *XP-1* is effective in the presence or absence of serum. It is also convenient; siPORT *XP-1*/DNA complexes can be added directly to growing cells in serum-containing culture medium, and the cells can be cultured until the time of assay without changing or adding media. A current listing of cell lines that have been successfully transfected using siPORT *XP-1* is posted on our web site at the following address:

www.ambion.com/catalog/CatNum.php?4506

B. Background and Applications

siPORT *XP-1* is designed for transfection of DNA, such as plasmids and PCR products, into cultured mammalian cells for RNAi experiments.

Small interfering RNAs (siRNAs) are short, double-stranded RNA molecules that can target complementary mRNAs for degradation via a cellular process termed RNA interference (RNAi). RNAi is employed across many disciplines to analyze gene function in mammalian cells. RNAi can be induced via transfection of cells with plasmids or siRNA expression cassettes (SECs) containing RNA polymerase promoters upstream of hairpin siRNA templates. Once in the cell, endogenous RNA polymerases transcribe hairpin siRNA from these templates to induce an RNAi effect.

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C. Example Procedure for Transfection

The optimal conditions for transfecting plasmid DNA or PCR products into cells is highly dependent on the identity and condition of the DNA, and on the cells being transfected.

This example procedure is intended only as a starting point towards optimizing transfection reactions. To identify the optimal transfection procedure for your cells and your SEC DNA, you will need to test different amounts of siPORT *XP-1*, DNA, and cells. See section D on page 4 for suggestions on optimizing transfection. Table 2 shows reagent amounts per well scaled for different types of culture plates using a a 3:1 ratio of siPORT *XP-1* (µL) to plasmid DNA (µg) or a 6:1 ratio of siPORT *XP-1* (µL) to PCR-product DNA (µg). We have found this ratio to be optimal for a number of different cell types.

Table 1. Approximate Reagent Amounts per Well for Transfection of Adherent Cells in Different Culture Plates

	Culture Plate			
	96-well	24-well	12-well	6-well
Well size (cm^2)	0.32	1.9	3.8	9.4
Cell plating (per well)	$0.2–1 \times 10^4$	$1.2–6 \times 10^4$	$0.25–1 \times 10^5$	$0.6–2.5 \times 10^5$
siPORT *XP-1*	0.12 µL	0.6 µL	1.2 µL	3 µL
Dilute siPORT *XP-1* to:*	20 µL	50 µL	75 µL	100 µL
Plasmid DNA OR	40 ng	200 ng	400 ng	1 µg
PCR product	20 ng	100 ng	200 ng	500 ng
Cell culture volume	100 µL	500 µL	1 mL	2 mL

* We recommend OPTI-MEM I reduced serum medium (Gibco BRL) to dilute the siPORT *XP-1*.

The procedure below is an example for transfection of cells cultured in one well of a 24-well plate; it can be adapted for different culture plates by scaling volumes according to well size as shown in Table 1.

1. Plate cells and grow overnight

a. Approximately 24 hr before transfection, plate cells in normal growth medium (e.g. DMEM, 10% FBS) so that they will be 30–60% confluent after 24 hr. (See Table 1 for an estimate of how many cells to plate.)

b. Incubate overnight in normal cell culture conditions.

2. Prepare siPORT *XP-1*/DNA transfection complexes

These instructions give examples of reagent amounts to use ***per well*** in 24-well plates. When possible, prepare master mixes of the reagents for transfection complex formation to minimize variability. Before use, allow the siPORT *XP-1* to equilibrate to room temp, and invert the tube or gently vortex to mix.

a. In a sterile, round bottom (or V-bottom) 96-well plate or in sterile polystyrene tubes, dilute 0.3–1.2 µL siPORT *XP-1* dropwise into Opti-MEM I medium (Gibco BRL) for a final volume of 50 µL.

b. Vortex thoroughly, incubate at room temp 5–20 min.

c. Add 200 ng of plasmid DNA or 100–200 ng of PCR product to the diluted siPORT *XP-1*, mix by gently flicking the tube or pipetting.
(optional) For some cell types, including carrier DNA (e.g. *Sau*3 A digested pUC 19 DNA, or salmon sperm DNA), in addition to the PCR product, can reduce the amount of PCR product required for optimal transfection efficiency. See section *D. Using carrier DNA* on page 6 for more information. This option should be tested as part of the transfection optimization process.

d. Incubate at room temp for 5–20 min.

3. Transfection

Separate instructions are provided below for transfection in culture media containing serum, and in serum-free media using siPORT *XP-1*. Follow the instructions in section 3a or 3b below depending on whether your medium contains serum.

3a. Transfection in media with serum (24-well plate)

a. If necessary, adjust the volume of normal growth medium in each well containing cells to 500 µL.

b. Overlay the siPORT *XP-1*/DNA complex (from step C.2.d above) dropwise onto the cells.

c. Without swirling, gently rock the dish back and forth to evenly distribute the complexes.

d. Incubate 24–72 hr in normal cell culture conditions, then assay for target gene activity.

3b. Transfection in serum-free media (24-well plate)

a. Remove the normal growth medium, and wash cells briefly with 1 mL serum-free medium.

b. Remove the wash medium, and add 500 µL fresh serum-free medium to the cells.

c. Overlay the siPORT *XP-1*/DNA complex (from step C.2.d above) dropwise onto the cells.

d. Without swirling, gently rock the dish back and forth to evenly distribute the complexes.

e. Incubate 2–8 hr in normal cell culture conditions.

f. Remove the medium containing siPORT *XP-1*/DNA complex, and replace it with normal growth medium (e.g. DMEM, 10% FBS).

g. Incubate 24–72 hr in normal cell culture conditions, then assay for target gene activity.

D. Transfection Optimization

Some of the most important parameters for maximizing transfection efficiency while minimizing cytotoxicity are listed below.

Cell culture conditions

Healthy, proliferating cells transfect better than poorly maintained or otherwise unhealthy cells (e.g. mycoplasma contaminated). The recommended cell density for most cell types is 30–60% confluence on the day of the experiment. Table 1 pro-

vides plating densities that will result in 30–60% confluence after a 24 hr incubation for most cell lines. At this confluency, most cell cultures will be actively proliferating, however, the optimal plating density for efficient transfection will depend on the size, growth rate, and condition of the cells. Therefore, cell plating density should be determined empirically for each cell type and carefully maintained from experiment to experiment for reproducibility.

DNA quality

DNA should be free of degradation products and other contaminants including endotoxins.

Ratio of siPORT *XP-1* to DNA, and absolute amounts of siPORT *XP-1* and DNA

Two very important parameters to investigate for transfection optimization are the ratio of siPORT *XP-1* (µL) to DNA (µg), and the amounts of both of these reagents. The ratios of 3:1 for plasmid DNA and 6:1 for PCR product DNA were found to be optimal with most cell types at Ambion, but ratios from 1.5:1 to 32:1 may provide the best transfection efficiency and lowest cellular toxicity in your system. To optimize transfection for your DNA and cells, test different ratios of siPORT *XP-1* to DNA, and different amounts transfection agent and DNA as well. Table 2 provides suggested amounts of siPORT *XP-1* and DNA to use for initial optimization experiments using cell cultures in 24-well plates.

Table 2. Suggested siPORT™ *XP-1* and DNA Ratios and Amounts for Initial Optimization Experiments

DNA type	Ratio	siPORT *XP-1* (µL): DNA (ng)
Plasmid DNA	2:1	0.4 µL : 200 ng
	3:1	0.6 µL : 200 ng
	4:1	0.8 µL : 200 ng
	3:2	0.6 µL : 400 ng
PCR Product DNA	3:1	0.3 µL : 100 ng
	6:1	0.6 µL : 100 ng
	12:1	1.2 µL : 100 ng
	12:2	1.2 µL : 200 ng

Analyze transfection efficiency and cellular toxicity after transfecting your cells with the amounts and ratios of siPORT *XP-1* and DNA shown in Table 2.

- Based on your results, consider testing either higher or lower ratios of siPORT *XP-1* to DNA.
- If using more DNA (i.e. the 3:2 or 12:2 ratio shown in the table) gave the best results, repeat the experiment using the same ratios, but higher absolute amounts of both siPORT *XP-1* and DNA.
- Choose the ***ratio*** of siPORT *XP-1* to DNA and the absolute ***amount*** of both reagents that give the highest level of silencing, without being toxic, for use in future experiments. (Trypan blue exclusion or other cell viability assays can be used to monitor toxicity.)

Using carrier DNA

For cell lines where a relatively large amount of DNA is required for optimal transfection, it may be desirable to replace some of the PCR product with carrier DNA for transfection complex formation in step C.2 on page 3. Carrier DNA, such as molecular weight markers or salmon sperm DNA, can be used to maintain a constant siPORT *XP-1* to DNA ratio, and the amount of PCR product can be varied to find the minimum amount needed to induce the desired level of gene silencing. For instance, you might find that 100 ng of carrier DNA plus 100 ng PCR product provide the same level of silencing as 200 ng PCR product. Note that it is important to maintain the optimal siPORT *XP-1* to DNA ratio. An optimized ratio produces a transfection complex with a net charge suitable for optimal association with the cell surface.

Transfection in serum-supplemented or serum-free media

siPORT *XP-1* is an effective transfection agent in media both with and without added serum. In most cases we recommend trying transfection in media containing serum because it provides conditions for optimal cell proliferation. Some cell types, however, are sensitive to serum during transfection (i.e. there will be elevated cytotoxicity in media containing serum compared to serum-reduced or serum-free medium). Researchers

may also want to avoid serum-supplemented media for specialized applications where component(s) of serum affect expression of the genes being analyzed. Regardless of your choice of medium for use during transfections, always use serum-reduced medium (we recommend Opti-MEM I) to prepare transfection complexes.

Streamlining the transfection procedure

It may be possible to streamline the transfection procedure as follows (this alternative procedure has worked well for several cell types in experiments at Ambion). This streamlined procedure substantially reduces set-up time by eliminating the pre-plating step.

a. Begin preparing transfection complexes according to the instructions in section C.2 on page 3.

b. Meanwhile, trypsinize a healthy actively growing culture, count the cells, and dilute them in culture medium so they will be at ~50% confluency when plated.

c. Dispense transfection complexes into the wells of a fresh culture plate.

d. Immediately plate cells into the wells of the plate containing transfection complexes from step c above.

e. Incubate 24–72 hr in normal cell culture conditions, then assay for target gene activity.

E. Troubleshooting

1. Low transfection efficiency

a. Optimize the following:

- siPORT *XP-1* to DNA ratio: See section *D. Ratio of siPORT XP-1 to DNA, and absolute amounts of siPORT XP-1 and DNA* on page 5
- Cell density: Maintaining a dividing cell culture is important for ensuring high transfection efficiency. Vary cell density around the values shown in Table 1 on page 2 to determine the optimal plating density for your cells.

- Analysis: Vary the time from transfection to measurement of gene expression. Typically 24–72 hr is optimal for analysis of gene silencing, but we suggest incubating for at least 72 hr to measure a reduction in protein knockdown.

b. Poor quality of the DNA being transfected

- DNA is degraded or contaminated with impurities or endotoxins.
- Double-check DNA concentrations and analyze on an agarose gel.

c. Suboptimal cell culture conditions

- ***Use healthy cells:*** Routinely subculturing cells before they become overcrowded or unhealthy will minimize instability in continuous cell lines and ensure an actively dividing cell population.
- ***Monitor cell passage history:*** Cells may have been subcultured too many times or have undergone changes. Transfect cells within 20 passages of optimization experiments.

d. siPORT *XP-1*/DNA complex didn't form properly

Follow the instructions for transfection complex formation closely; using the appropriate incubation times is important for good transfection efficiency.

Serum, polyanions, or other inhibitors were present during complexing.

Use OPTI-MEM I reduced-serum medium for transfection complex formation.

Do not overmix.

It is important to mix the DNA with the diluted transfection agent ***gently*** in step 2.c on page 3.

e. Inactivated transfection agent

Store siPORT *XP-1* at 4°C. Do not allow it to warm to temperatures above ~25°C (room temp).

If siPORT *XP-1* is accidentally stored at –20°C, warm it to room temp and gently vortex to redissolve any precipitate prior to use.

2. Transfection Causes Extensive Cell Death

a. Too much transfection agent was used

Use the smallest amount of transfection agent that still gives good transfection efficiency.

b. Cells were exposed to siPORT *XP-1*/DNA transfection complex for too long

Sensitive cells may begin to die from exposure to the siPORT *XP-1*/DNA transfection complex after a few hours. If transfection causes excessive cell death with your cells, remove the transfection mixture, and replenish with fresh growth medium after 2–24 hr.

c. Cells are stressed

- Avoid using antibiotics when plating cells for transfection, and for at least 72 hr after transfection.
- Use healthy cells that have been routinely subcultured before the point of medium depletion.
- Avoid subjecting cells to frequent temperature shifts.

d. The gene targeted by the siRNA is critical for cell survival

If the target gene is critical for cell survival, reducing its expression could cause cell death. If this is the case, assay mRNA levels at earlier time points (4–24 hr) after transfection.

e. Cell density is too low

Optimize cell density around the values provided in Table 1 on page 2 to determine the optimal plating density for your cells.

Some cells require cell-to-cell contact for optimal growth, these cell should be plated at higher cell densities (~50–60% confluency)

3. Experiments lack reproducibility

a. Transfection complex was not properly mixed with cells

After adding the siPORT *XP-1*/DNA complex to the cells, distribute them by gently rocking the plate back and forth. Do not swirl plates to mix, because this will concentrate reagents in the center of the wells.

b. There were differences in the experimental procedure

The time of transfection after cell plating, incubation times, master mix volumes, temperature, pH of media, and the order of component addition can all have subtle effects on transfection. To obtain reproducible results in gene silencing experiments, be very careful to conduct experiments the same way every time.

c. Cells passage number is too high

Repeat experiment using cells that have been subcultured fewer times.

F. siPORT™ *XP-1* Specifications

Contents

Amount		Storage
1 mL	siPORT *XP-1* Transfection Agent	4°C

Functional quality control testing

Cells were transfected with an eGFP reporter containing plasmid using siPORT *XP-1*. Cells transfected with the eGFP reporter containing plasmid are shown to have at least 50% efficiency in eGFP expression.

G. Safety 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.

Obtaining the MSDS

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

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