RNA Interference Research Guide

Applied Biosystems

Updated Version

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Introduction to RNAi and siRNAs

RNA interference, the biological mechanism by which double-stranded RNA (dsRNA) induces gene silencing by targeting complementary mRNA for degradation, is revolutionizing the way researchers study gene function. For the first time, scientists can quickly and easily reduce the expression of a particular gene in nearly all metazoan systems, often by 90% or greater, to analyze the effect that gene has on cellular function. The ease of the technique, as well as the wide availability of high quality kits and reagents for performing RNAi experiments, has driven its incredibly rapid adoption by the research community.

The RNAi Mechanism

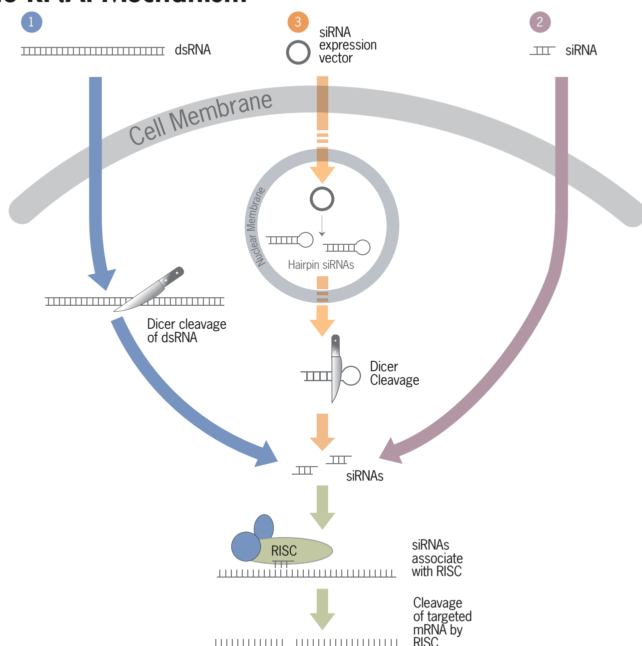


Figure 1. Three Ways to Trigger the RNAi Pathway. (1) In non-mammalian systems, the RNAi pathway commences when double-stranded RNA (dsRNA; usually longer than 30 bp) is introduced into cells. In mammalian systems, RNAi can be triggered by synthetic short interfering RNA (siRNA) molecules (2) or by DNA based expression vectors designed to express short hairpin RNA (shRNA) molecules (3). In each case, gene silencing results from destruction of mRNA that is complementary to the input siRNA (2) or the siRNA molecules created by Dicer cleavage of longer dsRNA (1) or shRNA (3) molecules. See text for additional details. Dicer=cytoplasmic nuclease; RISC=RNA-induced silencing complex; mRNA=messenger RNA.

In many non-mammalian systems, introducing or expressing long double-stranded RNA (dsRNA) triggers the RNAi pathway (Figure 1, pathway 1). The cytoplasmic nuclease Dicer first cleaves the long dsRNA into 21–23 bp small interfering RNAs (siRNAs), that then unwind and assemble into RNA-induced silencing complexes (RISCs). The antisense siRNA strand then guides RISC to complementary RNA molecules, and RISC cleaves the mRNA, leading to specific gene silencing.

Since most mammalian cells mount a potent antiviral response upon introduction of dsRNA longer than 30 bp, researchers induce RNAi in these systems by either transfecting cells with siRNAs (typically 21 bp RNA molecules with 3' dinucleotide overhangs) or by using DNA-based vectors to express short hairpin RNAs, (shRNAs), that are processed by Dicer into siRNA molecules (Figure 1, pathways 2 and 3, respectively).

Reagents Needed for RNAi Experiments

The required reagents for RNAi experiments are really quite simple. You need:

1. a dsRNA (i.e., siRNA or long dsRNA) that is completely complementary to the gene transcript(s) you wish to target by RNAi
2. a means to deliver that dsRNA to cells
3. proper controls
4. a way to detect the biological effect of reducing target gene expression (i.e., an assay).

Non-mammalian RNAi Experiments

In non-mammalian systems such as *Caenorhabditis elegans* and *Drosophila*, long dsRNA (e.g., typically >200 bp) complementary to the target transcript is used to induce RNAi. The dsRNA can be readily generated by in vitro transcription, and the MEGAscript® RNAi Kit (page 37) was developed specifically for this purpose. For genome-wide RNAi screening in *Drosophila* cells, the *Silencer*® *Drosophila* RNAi Library (page 38) provides ready-to-use dsRNA corresponding to 13,071 *Drosophila* genes.

siRNA Design and Synthesis for Mammalian RNAi Experiments

In mammalian cultured cells, RNAi is typically induced by the use of short interfering RNAs. siRNAs are generally 21 bp double-stranded RNA molecules with dinucleotide 3' overhangs. They can be introduced directly by transfection or electroporation, or generated within the cell from a short hairpin RNA expressed from a DNA construct. Good experimental design dictates that at least two functional siRNAs to the same target should be used independently to ensure that the biological effect is due to silencing of the target gene and not due to an off-target effect.

The most common way to prepare siRNAs is by chemical synthesis. siRNAs also can be expressed as hairpin structures from plasmid or viral vectors. Each method has its pros and cons (see page 6). For instance, chemically synthesized siRNAs are easiest to use and are easy to transfect in many cell types, whereas expression of siRNAs from DNA constructs permits long-term studies when an appropriate plasmid or viral vector is chosen. Both require careful design of the siRNA to maximize silencing of the target while minimizing off-target effects.

Ambion provides expert-designed, guaranteed-to-silence siRNAs for all human, mouse, and rat genes. These ready-to-use, chemically synthesized siRNAs are available individually as *Silencer*® Pre-designed siRNAs or Silencer Validated siRNAs, and in genome wide and functional class-focused sets as Silencer siRNA Libraries. Individual siRNAs allow detailed analysis of an individual gene's role in one or more pathways, whereas siRNA libraries, or sets of siRNAs targeting a pre-defined or custom set of genes, enable large scale screening experiments to correlate genes with cellular function. Learn more on pages 16–21.

Delivery of siRNAs

Once you have an siRNA, you need a means to deliver it—siRNA delivery conditions need to be tested and optimized for each cell type being used. For many immortalized cell lines, transfection with a lipid- or amine-based reagent is the preferred option. Delivery into primary cells and suspension cells, however, can be problematic if not impossible using standard transfection methodologies. In these cases, electroporation using a specialized, gentle-on-cells buffer, such as siPORT™ siRNA Electroporation Buffer, and optimized pulsing conditions generally results in very efficient siRNA delivery without compromising cell viability. See pages 10–11 for details on siRNA delivery and its optimization. siRNAs can also be used in vivo, and great progress has been made in this area. Because the in vivo RNAi field is moving quickly, Ambion provides up-to-date information in The RNAi Resource at **www.ambion.com/RNAi.**

Controls for siRNA Experiments

Proper controls are needed for every experiment, and RNAi experiments are no different. A negative control that does not target any endogenous transcript is needed to control for nonspecific effects on gene expression caused by transfecting any siRNA. Positive control siRNAs to easy-to-assay targets are needed to optimize transfection conditions, ensure that siRNAs are efficiently delivered, and ascertain that a particular downstream assay is working. Finally, fluorescently labeled control siRNAs facilitate the monitoring of siRNA delivery efficiency. See pages 12–13 for further details.

Assay for RNAi Effect

Assays that measure gene silencing and its effects are varied and diverse. For understanding the biological effects of knocking down a target gene, cell-based assays, enzymatic assays, array analysis, and countless other tools can be used. But before those assays can be run, a researcher needs to confirm that the siRNA is inducing knockdown of its intended target.

siRNAs exert their effects at the mRNA level. Therefore, the preferred assay for siRNA validation is one that monitors target mRNA levels. The simplest and most sensitive assay for siRNA validation relies on qRT-PCR to measure target transcript levels in gene specific siRNA-treated cells versus negative control siRNA-treated cells. Applied Biosystems' TaqMan® Gene Expression Assays (page 40) are ideal for this purpose. Ambion's siRNA database (**www.ambion.com/siRNA**) provides links to individual assays matched to gene specific *Silencer* Pre-designed siRNAs and *Silencer* Validated siRNAs, which makes finding both siRNAs and real-time PCR assays to a particular gene simple.

Although it is necessary to monitor mRNA levels to validate siRNAs, many researchers also wish to determine the extent of knockdown at the protein level. Western blotting, immunofluorescence and flow cytometry are typically used for this purpose. Often time course experiments are needed to find the points of maximal mRNA and protein knockdown.

Many researchers performing experiments in animals will wish to correlate siRNA, target mRNA, and target protein levels. Isolation and detection of small RNAs, however, requires modified, and in some cases, completely different techniques than isolation and detection of longer RNAs. For more information on kits and reagents specifically for siRNA, miRNA and other small RNA analysis, see the miRNA Resource at **www.ambion.com/miRNA.**

1 Visit www.ambion.com/siRNA and order:
- Three *Silencer*® Pre-designed or Validated siRNAs per Target
- siPORT™ Transfection Agent or Electroporation Buffer
- *Silencer*® siRNA Controls

2 Plate cells and transfect siRNAs

3 Monitor siRNA-induced knockdown to:
- Validate the siRNA
- Monitor transfection efficiency
- TaqMan® Gene Expression Assays

3 Observe/Measure Phenotypic Change

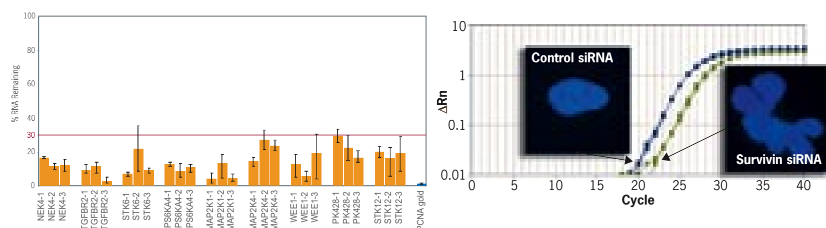


Figure 2. RNAi Experiment Workflow Using siRNAs in Cultured Cells.

More Information

For detailed information about RNAi, including protocols, products and the latest tips and techniques, visit:

The RNAi Resource
www.ambion.com/RNAi

Ambion RNAi Products and siRNA Database
www.ambion.com/siRNA

TaqMan® Gene Expression Asssay Database
www.allgenes.com

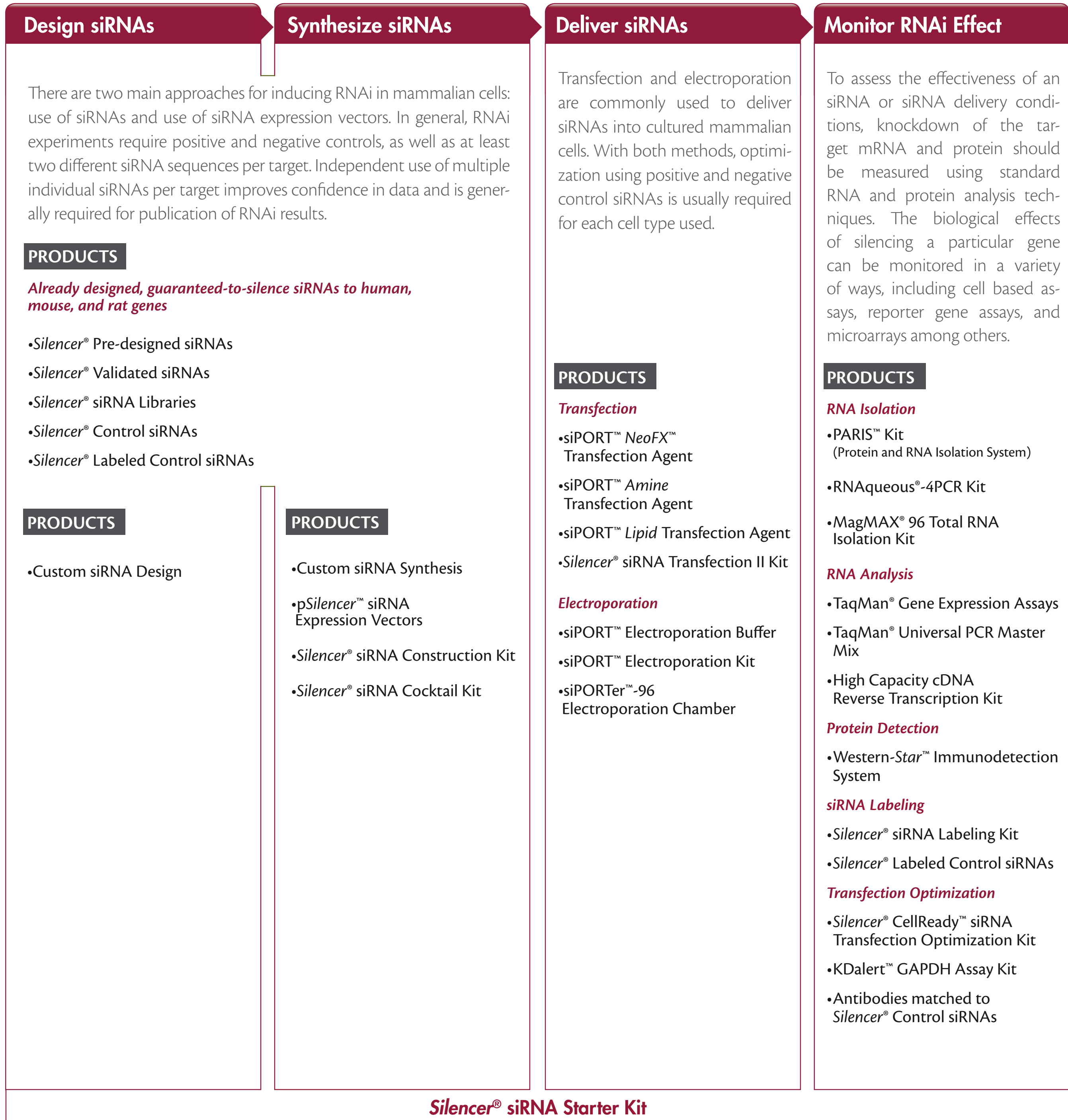
siRNA Delivery Resource
www.ambion.com/siRNA/delivery

In Vivo siRNA Resource
www.ambion.com/siRNA/invivo

TaqMan® is a registered trademark of Roche Molecular Systems, Inc.
Applied Biosystems® is a registered trademark of Applera Corporation.

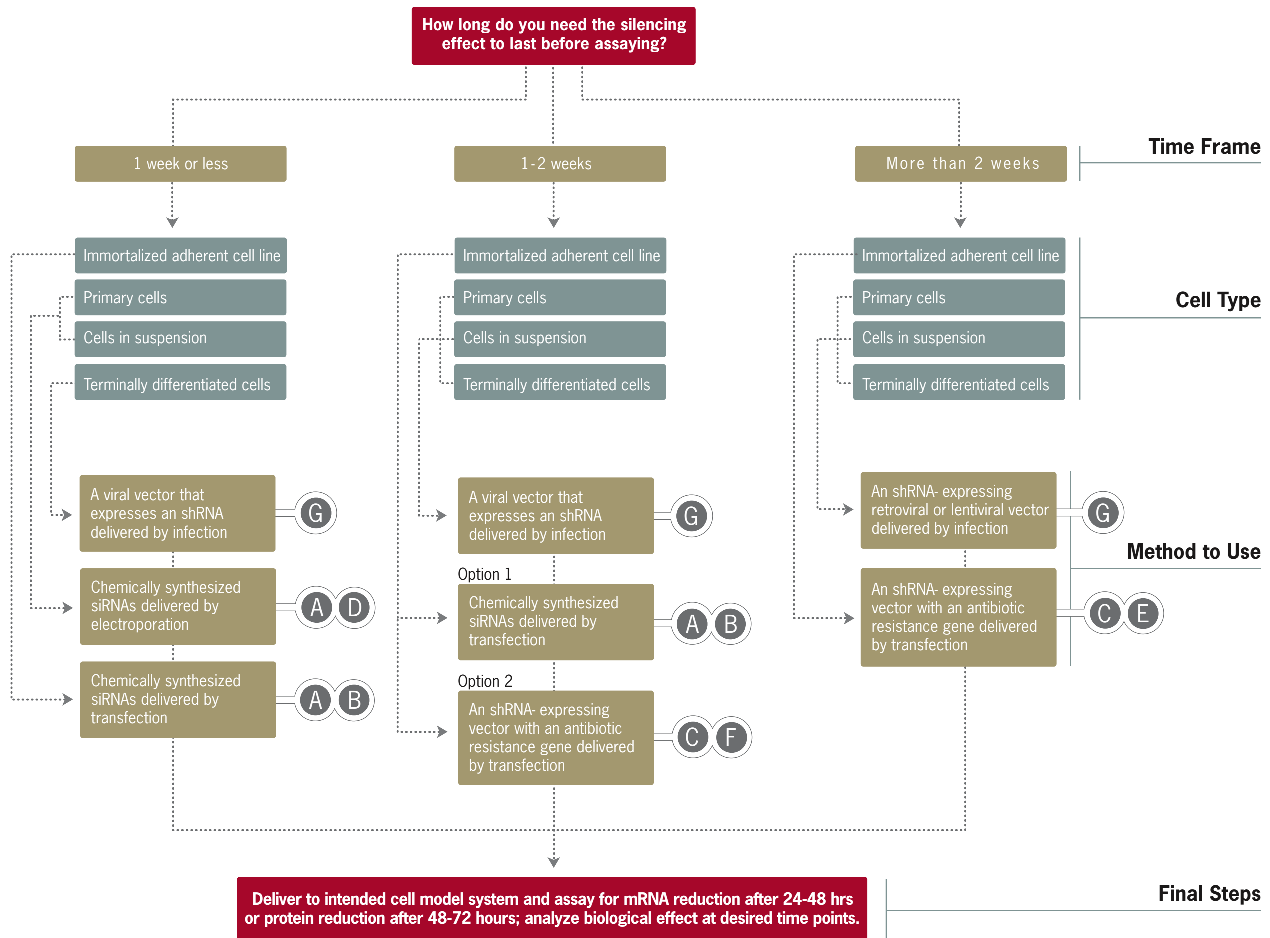
Inducing RNAi in Mammalian Cultured Cells: Experimental Overview

Since 2001, Ambion scientists have conducted thousands of siRNA experiments. The result is unrivalled expertise in siRNA design, synthesis, delivery, and detection—as well as the broadest range of RNAi products available anywhere. Below is a quick overview of the products discussed in this guide. Details can be found inside and on the web at **www.ambion.com/RNAi.**



siRNA Decision Tree: A Guide to Designing siRNA Experiments

Determining which approach to use for an RNAi experiment in mammalian cultured cells begins with answering a few simple questions. The first question is whether you need long-term silencing. Transfection of siRNAs usually induces strong silencing for 3–7 days; the length of the effect depends on the proliferation rate of the cells and the potency of the siRNA. Most experiments can be completed in this amount of time. Although you can re-transfect cells to extend the RNAi effect, if your experiment requires more than a week of silencing, a vector-based approach may be a better choice. An interactive and more detailed version of this decision tree is available on the Ambion website at **www.ambion.com/RNAi.**



A

Use:
- *Silencer*® Pre-designed siRNAs
- *Silencer*® Validated siRNAs
- *Silencer*® Control siRNAs
- *Silencer*® siRNA Libraries
- Custom siRNA Synthesis

B

Use:
- siPORT™ *NeoFX*™ Transfection Agent
- siPORT™ *Amine* Transfection Agent

Optimize with:
- *Silencer*® Transfection II Kit
- *Silencer*® CellReady™ Transfection Optimization Kit
- *Silencer*® Control siRNAs

C

Express with:
- p*Silencer*™ 2.1-U6
- p*Silencer*™ 3.1-H1
- p*Silencer*™ 4.1-CMV

D

Use:
- siPORT™ Electroporation Buffer/Kit
- siPORTer™ - 96 Electroporation Chamber

Optimize with:
- siPORT™ Electroporation Kit
- *Silencer*® Control siRNAs
- *Silencer*® Labeled Control siRNAs

E

Try:
- siPORT™ Electroporation Buffer for plasmid delivery

F

Try:
- siPORT™ *XP-1* for delivery

G

Express with:
- p*Silencer*™ 5.1 Retro
- p*Silencer*™ adeno1.0-CMV System (transient only)

siRNA Design: Ambion Answers Your Questions

Experiments at Cenix BioScience, Ambion, and elsewhere [1,2] have shown that design rules exist for siRNAs, and that they can be exploited to both improve the chances that an siRNA will be active and enhance the efficacy of the siRNAs. Ambion uses a sophistcated algorithm to design *Silencer*® Pre-designed siRNAs, *Silencer* Validated siRNAs, and *Silencer* siRNA Libraries. Here we answer some frequently asked questions about the algorithm.

How Does the Algorithm Compare to Publicly Available siRNA Design Programs?

Publicly available siRNA design programs typically show success rates of only 50–60% in generating siRNAs that yield over 70% silencing of target mRNA levels in HeLa cells 48 hrs post transfection. The algorithm used by Ambion results in success rates that exceed 82% for producing effective siRNAs.

How Effective is the Algorithm, and How Was Its Effectiveness Measured?

Initial tests of the algorithm's success rate were conducted by measuring silencing efficacy using quantitative RT-PCR to measure target mRNA levels in HeLa cells 48 hr after transfection. In one experiment, 79 algorithm-designed siRNAs, each of which targeted a different human transcript, were tested. The analysis revealed that 74 of the 79 siRNA, or 94% of the tested siRNAs, gave higher than 70% silencing (Figure 3).

In subsequent analyses, it was noted that certain genes, and in some cases, certain transcripts, appear significantly more refractory to siRNA-based silencing than the majority. Since the preponderance of these "tougher genes" remained unknown at the genome level, it became clear that any extrapolations of silencing success rates from tens or even hundreds of siRNAs would be of limited statistical value. Thus, the performance analysis was extended to more than 1,100 siRNAs targeting nearly 400 endogenously-expressed human transcripts (Figure 11, page 16). On a per siRNA basis, approximately 82% of the individual siRNAs showed >70% silencing of their target, and the vast majority of those silenced their target >85%. The performance data clearly confirm the success of Ambion's siRNA selection process.

How Potent Are the siRNAs Designed by the Algorithm?

The algorithm results in siRNAs that are remarkably potent (Figure 4). Using *Silencer*® siRNAs in optimized transfection systems, researchers routinely observe greater than 90% reduction in target mRNA levels with nM or even pM amounts of siRNA.

What Makes the Algorithm So Successful?

The algorithm incorporates several parameters to predict effective siRNAs. Early on, it was recognized that siRNAs containing higher G/C content at certain residues near the 3' end of the antisense strand, and lower G/C content at certain residues near the 5' end of the antisense strand offered significantly higher success rates. This principle was therefore implemented as one of the algorithm determinants for maximizing silencing efficacy. Since then, other researchers have suggested a biological reason for this observation—that the strand of the siRNA whose 5' end has lower G/C content is preferentially loaded into RISC [1].

In addition to G/C content at the termini of the siRNAs, traits that were found to be influential in defining optimal siRNA sequences include the T_m of specific internal domains of the siRNA, siRNA length, position of the target sequence within the coding sequence and nucleotide content of the 3' overhangs. These parameters along with several others are incorporated into the algorithm, and the combination leads to a high success rate.

How Does the Algorithm Ensure Specificity of an siRNA?

The design process includes a stringent specificity check whereby both siRNA strands are subjected to customized alignment searches to minimize risks of generating off-target effects. Putative siRNA target sequences are also screened against the most updated single-nucleotide polymorphism (SNP) databases to avoid variability. Target mRNA sequences known to mediate regulatory processes through binding to protein factors are similarly avoided, as are CpG motifs.

References

1. Schwarz D, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD (2003) Asymmetry in the assembly of the RNAi enzyme complex. *Cell* **115(2):** 199–208.
2. Khvorova A, Reynolds A, Jayasena JD (2003) Functional siRNAs and miRNAs exhibit strand bias. *Cell* **115(2):** 209–216.

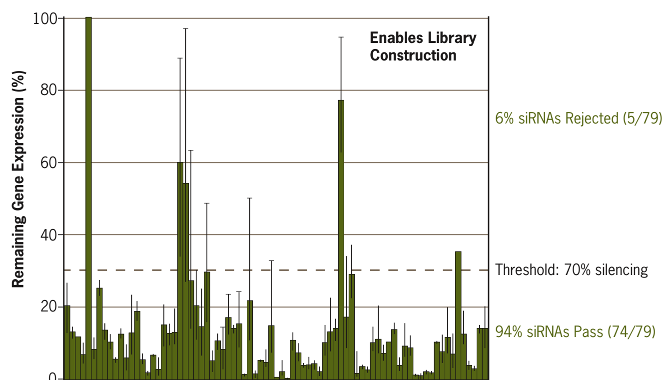


Figure 3. Silencing Efficacy of 79 Algorithm-designed siRNAs Targeting 79 Endogenously Expressed Human Kinases. Target mRNA levels were measured by qRT-PCR and dC_t values were normalized against 18S rRNA. Samples were harvested 48 hr after siRNA transfection into HeLa cells. Data courtesy of Cenix Bioscience.

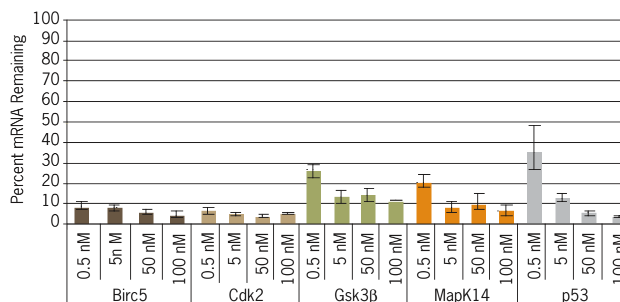
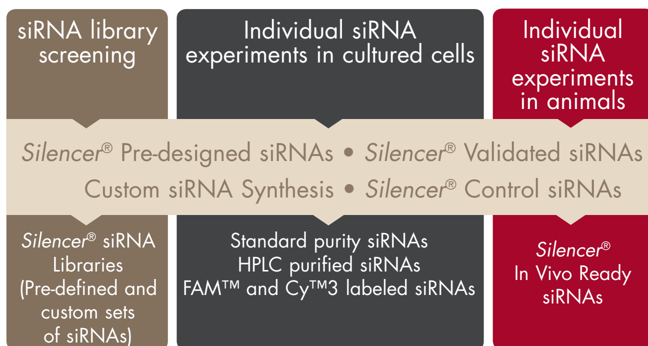


Figure 4. Potency of *Silencer*® siRNAs at Low Concentrations. siRNAs were transfected into HeLa cells at the indicated concentrations, and target mRNA levels were monitored by real-time RT-PCR 48 hr after transfection using the appropriate TaqMan® Gene Expression Assay. Transfections were performed in triplicate, and the data are shown relative to *Silencer* Negative Control #1 siRNA transfected cells.

Overview of Ambion siRNAs

- ***Silencer*® Pre-designed siRNAs** (page 16)—Guaranteed-to-silence siRNAs to essentially all human, mouse, and rat genes.
- ***Silencer*® Validated siRNAs** (page 17)—Individual siRNAs functionally tested in cells and guaranteed to reduce target gene expression.
- **Custom siRNAs** (page 18)—Premium quality siRNA synthesized to your specifications with a sequence you provide.
- ***Silencer*® siRNA Libraries** (page 20-21)—Sets of siRNAs—both large and small—to the human and mouse genomes. Custom sets available.
- ***Silencer*® Control siRNAs** (page 23)—Highly validated siRNAs for use as positive and negative controls.

Ambion provides custom siRNA synthesis, as well as expert-designed, guaranteed-to-silence siRNAs for all human, mouse, and rat genes. These ready-to-use, chemically synthesized siRNAs are available individually and in pre-defined and customer-defined sets. Individual siRNAs allow detailed analysis



of an individual gene's role in one or more pathways, whereas siRNA libraries targeting a pre-defined or custom set of genes enable large-scale screening experiments to correlate genes with cellular function. In either case, Ambion provides the utmost in siRNA quality, convenience and service.

Description of siRNA Options

Standard Purity

Standard purity siRNAs are the most popular choice for experiments in cultured cells. After synthesis, each siRNA strand is cartridge-purified using a proprietary process that typically yields >90% full length oligo (>80% guaranteed). Prior to annealing, each strand is analyzed by MALDI-TOF mass spectrometry, and many by analytical HPLC. The efficiency of annealing is then verified by PAGE. Ambion uses standard purity siRNAs in our *Silencer*® siRNA Libraries. Standard purity is exclusively available for 21mer, unmodified siRNAs.

HPLC Purified

HPLC purification results in a greater percentage of full-length siRNA products than cartridge purification, and is a more efficient process for purifying large amounts of siRNA. HPLC purification is usually the method of choice for purifying siRNAs that are required in amounts >40 nmol, not 21 nt in length, or are modified. Ambion's *Silencer* Control siRNAs and In Vivo Ready siRNAs are HPLC purified. HPLC-purified siRNA strands are analyzed by MALDI-TOF mass spectrometry and analytical HPLC. The efficiency of annealing is verified by PAGE.

PAGE Purified

Polyacrylamide gel electrophoresis (PAGE) gives better resolution than HPLC for oligonucleotides longer than ~30 nt, and therefore results in higher purity when purifying oligos >30 nt in length. For most 21mer siRNAs, the difference in purity between PAGE and HPLC purification is minimal. PAGE purified siRNA strands are analyzed by MALDI-TOF mass spectrometry and analytical HPLC. The efficiency of annealing is verified by PAGE.

In Vivo Ready

Ambion's In Vivo Ready siRNA option provides high quality siRNAs that are ready for introduction into animals. Each siRNA strand is purified by HPLC, subjected to an additional dialysis step to remove salt, and then endotoxin tested. Of course each siRNA strand is also analyzed by MALDI-TOF mass spectrometry and analytical HPLC, and the efficiency of annealing is verified by PAGE.

FAM™ and Cy™3 Labeled

Cy3 and FAM Dye labeled siRNAs are labeled at the 5' end of the sense strand during chemical synthesis. Modification of this site has no significant effect on siRNA efficacy, and the labels can be used to detect siRNAs within cells via fluorescence microscopy or flow cytometry. After synthesis, each siRNA strand is HPLC purified and subjected to MALDI-TOF mass spectrometry and analytical HPLC analysis. The efficiency of annealing is verified by PAGE.

Table 2. Excitation and Emission Wavelengths of FAM™ and Cy™3 Dyes.

Label	Color	Excitation Maximum	Emission Maximum
Cy™3 Dye	Orange	547 nm	563 nm
FAM™ (a derivative of fluorescein) Dye	Green	494 nm	520 nm

For Research Use Only. Not for use in diagnostic procedures.

Table 1. Purification Options for Pre-designed, Validated and Custom siRNAs.

Type	Recommended Use	Quality Specifications				
		Minimum Purity Level	MALDI-TOF Mass Spec	Analytical HPLC	PAGE Quality Check of Annealing	Dialyzed, Filter Sterilized, and Endotoxin Tested
Standard Purity	Cell culture	Average >90% pure (guaranteed >80%)	All	Some	All annealed siRNAs	No
HPLC Purified	Cell culture when you need large quantities of material. Also used on siRNAs with modifications or nonstandard-length strands	>97% pure	All	All	All annealed siRNA	No
In Vivo Ready	Animals	>95% pure	All	All	All annealed siRNA	Yes

How to Order siRNAs from Ambion

Silencer® Pre-designed and Validated siRNAs

Guaranteed silencing with siRNAs to all human, mouse, and rat genes (see page 16–17)

1) Go to **www.ambion.com** and click on “siRNA Database”

2) Search by gene name, accession number, functional class, or siRNA ID.

3) Select the siRNAs you want and click “Copy Checked Items to Clipboard”. (Note the convenient link to associated TaqMan® Gene Expression Assays.)

4) Choose options.
Follow the instructions to order online, or by fax or e-mail.

Custom siRNA

Premium quality siRNA synthesized with the sequence you specify

1) Go to **www.ambion.com/catalog/siRNA_order.html**

2) Follow instructions to input desired siRNA sequence.
3) Choose options.
4) Follow the instructions to order online, or by fax or e-mail.

Silencer siRNA Libraries

Sets of siRNAs to the human genome and human, mouse, and rat functional gene classes. Custom sets available. See page 20–21 for details.

Due to their customizable nature, most *Silencer* siRNA Libraries are provided using a custom quoting process. You can request a quote using the form at **www.ambion.com/info/libraries** or via email at **libraries@ambion.com**.

siRNA Delivery – Ambion Answers Your Questions

How Do I Deliver siRNAs Into Cultured Mammalian Cells?

The two most commonly used methods for delivering chemically synthesized and in vitro transcribed siRNAs into cultured cells are lipid mediated transfection and electroporation.

Some cell types, including most adherent immortalized cell lines, can be readily transfected with lipid based transfection agents and methods developed for use with siRNA. Other cell types, such as some finite cell lines, freshly isolated primary cells, and cells grown in suspension, present more of a challenge. Electroporation in the presence of the right buffer can very successfully deliver siRNA into these cells. Both delivery methods require optimization for best results.

How Should I Monitor siRNA Delivery?

To monitor siRNA delivery and to optimize transfection, you should transfect a positive, verified-to-silence control siRNA side by side with a non-targeting, negative control siRNA and then assess delivery efficiency. Cell viability should also be measured in negative control transfected cells versus non-transfected cells. There are four possible ways to monitor siRNA transfection efficiency using control siRNAs:

1. Monitor target mRNA knockdown
2. Monitor target protein knockdown
3. Monitor an induced phenotype
4. Label the siRNA and monitor uptake by fluorescence microscopy or flow cytometry

Because siRNAs exert their effects at the mRNA level, monitoring target mRNA levels by real-time PCR is an excellent method for assessing siRNA delivery efficiency. Applied Biosystems TaqMan® Gene Expression Assays are ideal for this purpose. For instance, Ambion's *Silencer*® GAPDH siRNA induces GAPDH mRNA knockdown of >95% when efficiently delivered into cells. If target mRNA levels are reduced to levels approaching 95% knockdown, you know that the siRNA was efficiently delivered and it is effectively inducing RNAi.

Protein levels, assessed by Western blot, FACS, immunofluorescence, or enzymatic assay, can also be used to assess siRNA delivery efficiency. Ambion's new KDalert™ GAPDH Assay Kit (page 24) uses a fluorescence based assay to rapidly and inexpensively assess GAPDH enzyme activity after transfection of GAPDH siRNA (the assay can also be monitored via spectrophotometry). This assay has an added advantage in that it can be used to monitor cell viability of negative control siRNA transfected cells versus non-transfected cells.

Some siRNAs, such as the *Silencer* KIF11(Eg5) siRNA, elicit a gross morphological change that can be monitored visually or with an image analysis program. In general, these types of controls and assays are more appropriate for confirming siRNA delivery rather than discerning quantitative differences in delivery efficiency as is needed for transfection optimization experiments.

Many researchers use fluorescently labeled siRNAs for transfection optimization procedures and then analyze siRNA uptake by fluorescence microscopy or flow cytometry. While rapid and inexpensive, apparent uptake of fluorescently labeled siRNA does not always correlate with induction of RNAi. The problem appears to be worse with certain transfection agents and cell types. In general, we do not recommend use of fluorescently labeled siRNAs for transfection optimization with lipid based reagents. However, if correlation between labeled siRNA uptake and target mRNA knockdown can be verified for a particular transfection agent and cell type, then labeled siRNAs can be used to verify siRNA delivery for that particular experiment.

Which Transfection Agent Should I Use?

Every transfection agent has a different delivery efficiency when used with different cell types. The overall transfection efficiency and degree of gene silencing depend on the nature of the transfection agent/siRNA complex. In general, we recommend testing a few transfection agents if you are trying to transfect siRNAs into a cell type for which you cannot find published siRNA delivery conditions.

Ambion provides two siRNA transfection agents, siPORT™ *NeoFX*™ Transfection Agent (page 29) and siPORT *Amine* Transfection Agent (page 30). Both reagents have been extensively tested and support efficient, reproducible siRNA transfection into a broad range of cell types, and both can be used with reverse transfection protocols. These two reagents are provided separately and in the *Silencer* siRNA Transfection II Kit (page 30), which also includes positive and negative controls for transfection optimization.

How Do I Optimize Transfection with Lipid Based Reagents?

Optimizing transfection efficiency while minimizing cytotoxicity is crucial for maximizing gene silencing. Optimal transfection efficiencies are achieved by identifying an effective transfection agent for each cell type and by adjusting (in order of importance):

- Amount of transfection agent (Figure 6, page 11)
- Amount of siRNA
- Cell density at the time of transfection
- Order of transfection (pre-plating cells or plating cells/transfecting simultaneously)
- Length of exposure of cells to transfection agent/siRNA complexes

The chart on page 11 provides helpful starting points for transfection of siRNA into cultured mammalian cells (see above for details on how to monitor siRNA delivery and pages 46–47 for additional cell type specific details).

	96 well	24 well	12 well	6 well
Reagent* (µL)	0.3–1.0	1–3	2–4	3–6
siRNA (pmol)**	3	15	30	75
Cell Density (cells/well)	6000	40,000	80,000	200,000
Final Volume/well (mL)	0.1	0.5	1.0	2.5

*Refer to the instructions provided with your transfection agent for the recommended volume of transfection agent.

**The siRNA amount shown results in an siRNA concentration of 30 nM. The amount of siRNA required for maximal gene silencing will vary from cell type to cell type.

Figure 5. Typical Amounts of Transfection Agents Required for siRNA Transfections.

The fastest way to optimize siRNA transfection in 96 well plates is to combine the *Silencer*® CellReady™ siRNA Transfection Optimization Kit (page 22)—which provides GAPDH and negative control siRNAs pre-plated into a 96 well plate; siPORT™ *NeoFX*™ Transfection Agent; and experimental templates for performing optimization experiments—with the KDalert GAPDH Assay Kit (page 24), which includes reagents and protocols for rapidly measuring GAPDH enzyme activity and cytotoxicity, and was developed specifically for transfection optimization experiments.

Once the conditions for maximal gene silencing are determined, they should be kept constant from experiment to experiment for a given cell type.

For more information about siRNA transfection, including conditions found to successfully deliver siRNAs into several common cell types, see pages 46–47 or visit Ambion's siRNA Delivery Resource at **www.ambion.com/siRNA/delivery**.

How Do I Deliver siRNAs to Primary and Difficult-to-transfect Cells?

Although they work well for transfecting siRNA into most immortalized adherent cell types, lipid based reagents do not efficiently deliver siRNAs into many primary cells or cells in suspension. For these cell types electroporation is a more efficient delivery mechanism.

Electroporation of siRNA is accomplished by suspending cells and siRNA together in a buffer and then applying one or more square wave type electrical pulses with an electroporator. The composition of the buffer is critical for success, as cell viability can be severely compromised if a suboptimal buffer is used. Ambion's siPORT Electroporation Buffer (page 31) was developed specifically for electroporating siRNA into a broad range of cell types. See page 47 for a table of recommended electroporation conditions for specific cell types.

How Do I Optimize Electroporation Parameters?

Optimal electroporation conditions are determined by adjusting:

- Voltage of pulse (typically 100V–500V)
- Pulse length (typically 100–800 µsec)
- Number of pulses (typically 1–3 pulses)

siRNA delivery can be followed by using a control siRNA such as GAPDH siRNA and then monitoring target mRNA or protein knockdown, or by using a Cy™3 dye labeled siRNA to assess siRNA delivery efficiency by fluorescence microscopy or flow cytometry. The siPORT siRNA Electroporation Kit is (page 31) combines siPORT siRNA Electroporation Buffer, a Cy3 dye-labeled control siRNA, a GAPDH control siRNA and a negative control siRNA—in other words everything you need for optimizing electroporation conditions.

Can I Electroporate Multiple Samples Simultaneously?

The siPORTer™-96 Electroporation Chamber (page 32), designed for use with the BioRad® Gene Pulser Xcell™ power supply, enables high throughput electroporation of siRNA libraries into primary cells, and can be used to screen large numbers of siRNAs rapidly, reproducibly, and in parallel. The combination of *Silencer* siRNA Libraries (see pages 20–21), siPORT siRNA Electroporation Buffer, and the siPORTer-96 Chamber provide an extremely effective approach for investigating gene function across entire gene classes.

Can I Use siRNAs in Animals?

Yes! siRNAs have been successfully used to silence targets in the liver, lung, brain, eye, kidney, skeletal muscle and tumors of rodents. There are also a handful of siRNA molecules in clinical trials in humans. Delivery routes and dosage schedules depend on the organ or tissue to be targeted. Most of the more than 100 published animal studies have been conducted with unmodified siRNAs delivered in buffer, however siRNAs modified to improve stability and/or encapsulated in a lipid or other nanoparticle have also been used. Ambion provides *Silencer*® In Vivo Ready siRNAs for use in animal studies (page 19). These siRNAs have been meticulously purified, dialyzed, and endotoxin tested to ensure their suitability for use in animals. For more information about the use of siRNAs in animals, including references and protocols, visit Ambion's In Vivo siRNA Resource at **www.ambion.com/siRNA/invivo**.

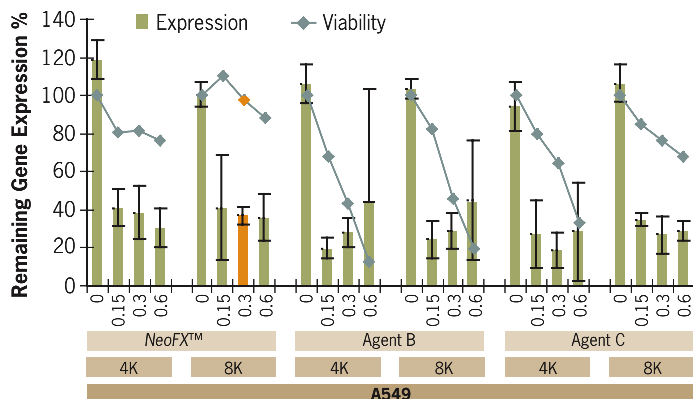


Figure 6. Optimization of siRNA Transfection Using the *Silencer*® CellReady™ siRNA Transfection Optimization Kit. Cell viability and knockdown of GAPDH levels in A549 cells was measured using the KDalert™ GAPDH Assay Kit 48 hours after the experiment was performed. The bar marked in orange represents the conditions eliciting efficient knockdown while maintaining high cell viability.

Getting the Most from Your RNAi Data: Which Controls to Use

Ambion scientists use and recommend a number of different controls for siRNA experiments. Most of these coincide with the suggested controls detailed in an editorial published in *Nature Cell Biology* [Whither RNAi? (2003) *Nature Cell Biology* **5**: 489–490].

Non-targeting, Negative siRNA Control

Because siRNA delivery methods induce changes in gene expression profiles, silenced experimental samples should be compared with cells transfected with a non-targeting, negative control siRNA rather than to non-transfected cell samples. Comparing cells transfected with such a "negative" siRNA control to untreated cells can reveal changes caused by siRNA delivery. *Silencer*® Negative siRNA Controls are highly validated negative control siRNAs that have limited sequence similarity to the human, mouse, and rat genomes. Ambion scientists, as well as many other scientists around the world, routinely use these negative controls in their RNAi experiments.

Positive siRNA Control for Monitoring siRNA Delivery

A positive siRNA control should be used in experiments to monitor, as well as to optimize, siRNA transfection efficiency. When the positive control fails to elicit the expected reduction in gene expression, poor transfection is immediately suspected. Ambion's *Silencer* GAPDH siRNA is a commonly used positive control because GAPDH is expressed at detectable levels in almost all cell types and GAPDH gene products are easy to assay. See page 23 for additional positive siRNA control options.

Multiple siRNAs to a Single Target

One concern among researchers using RNAi in mammalian cells is off-target effects. There are several reports of single siRNAs affecting the expression of multiple genes (Ambion's algorithm incorporates stringent specificity checks to minimize the potential for off-target effects). Overcoming this concern is a simple matter of confirming results with a second or third siRNA targeting a different region of the transcript being studied. The siRNAs should first be analyzed for effectiveness at reducing target gene expression. Different siRNAs to the same target with comparable gene silencing efficacy should induce similar effects. Any changes induced by one siRNA and not the other(s) could be attributed to off-target effects. One of the easiest ways to obtain multiple effective siRNAs to the same target is by searching Ambion's database of *Silencer* Pre-designed siRNAs. When you purchase three *Silencer* Pre-designed siRNAs to the same target, Ambion will guarantee that at least two of those siRNAs will knock down target mRNA levels by 70% or greater.

Monitor Both Target mRNA and Protein Levels

siRNAs trigger mRNA degradation through the RNAi pathway. In contrast, the closely related microRNAs usually inhibit translation without significantly affecting mRNA levels. Most researchers agree that it is important to monitor RNAi induced knockdown at both the mRNA and protein level. With an effective siRNA, you would expect to see reduction of both target mRNA and corresponding protein levels. mRNA reduction seen without a corresponding reduction in protein levels can indicate that protein turnover is slow—a different time point may be required for your biological assay. In contrast, protein reduction in the absence of mRNA reduction may indicate that an siRNA is mediating its effects at the translational level like a microRNA. To save time and conserve reagents, you can analyze mRNA and protein levels from a single sample. The PARIS™ Kit (Protein and RNA Isolation System), which provides a simple protocol to isolate native protein and total RNA from the same sample, makes this particularly straightforward.

Titrate the siRNA and Use the Lowest Possible siRNA Concentration

Several reports indicate that siRNA concentrations of 100 nM or higher in mammalian cultured cells can lead to nonspecific changes in gene expression [1, 2]. Using ≤30 nM of a highly effective siRNA appears to minimize nonspecific effects. Well-designed siRNAs, such as Ambion's *Silencer* Validated and Pre-

Why Use GAPDH Control siRNAs?

The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene is widely recognized as an ideal target for positive control siRNAs. GAPDH is expressed ubiquitously at fairly high levels in virtually all mammalian cells; this makes it extremely useful for evaluating both transfection efficiency and cell viability:

- Optimize transfection efficiency by transfecting a GAPDH positive control siRNA using experimental transfection agents and conditions to identify which provide the maximum knockdown of GAPDH activity.
- Optimize cell viability by transfecting a negative control siRNA to identify the transfection agent and conditions that do NOT cause knockdown of endogenous GAPDH activity and do not significantly decrease cell number compared to untransfected cells.

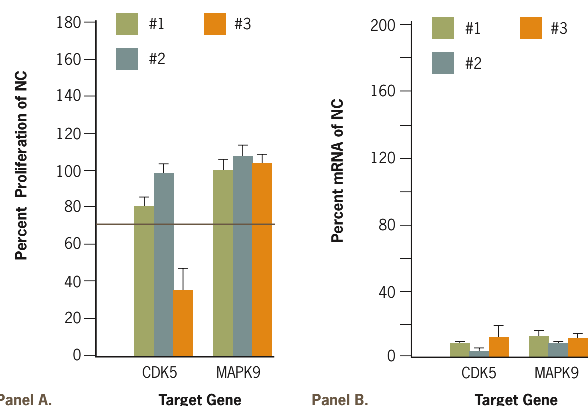


Figure 7. Importance of Confirming RNAi Effects with Multiple siRNAs. Three different siRNAs to CDK5 or MAPK9 were individually transfected in triplicate into HeLa cells at a final concentration of 30 nM. **(A)** 48 hours after transfection, relative cell number per well was determined using alamarBlue® (AccuMed International). **(B)** RNA was also isolated and assayed for target mRNA expression by real-time RT-PCR. Data are shown relative to cells transfected with *Silencer*® Negative Control #1 siRNA. Note that in the case of CDK5, cell numbers were dramatically reduced by siRNA #3, but not by the two other siRNAs to that target. This difference in biological effect cannot be explained by the difference in mRNA knockdown induced. These data illustrate the need for independently using multiple siRNAs to the same target to confirm silencing results.

designed siRNAs, can often be used at even lower concentrations, further reducing the chance of eliciting off target effects. Figure 12 on page 16 shows a titration experiment in which various concentrations of *Silencer*® Validated siRNAs were tested for their ability to knock down target mRNA levels. All of the siRNAs tested were effective at 10 nM, and five of the six were effective at 3 nM.

Controlling RNAi Screening Experiments

Monitoring both target mRNA and protein knockdown and perfoming titration experiments in large scale RNAi screens is time-consuming and often prohibitively expensive. At a minimum, one or more non-targeting, negative control siRNAs, as well as one or more positive control siRNAs to verify efficient siRNA delivery and the suitability of the assay chosen, should be used per plate analyzed. And validation of some or all of the "hits" using more extensive controls is warranted. See pages 14–15 for more information about ways to use siRNA libraries for both large and small scale RNAi screens. Ambion has also recently published an extensive guide to siRNA secreening. Request your free copy at **www.ambion.com/RNAi/screening**.

The use of RNAi has enormous potential for analyzing gene function, elucidating biological pathways, and identifying and validating potential drug targets. The RNAi field is still relatively new, and recommendations on experimental design and proper controls are likely to evolve. Ambion is committed to bringing researchers around the world the most up-to-date information on RNAi for their own research. For the latest information, see the RNAi resource at **www.ambion.com/RNAi.**

References

1. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G, Linsley PS (2003) Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol.* **21:** 635–637.
2. Semizarov D, Frost L, Sarthy A, Kroeger P, Halbert DN, Fesik SW (2003) Specificity of short interfering RNA determined through gene expression signatures. *Proc Natl Acad Sci* USA **100:** 6347-6352.

Top 5 Ways to Control Your siRNA Experiments

Recommended Control or Procedure	How to Accomplish
1. Check for nonspecific effects associated with siRNA delivery by transfecting a nonspecific negative control siRNA.	Use a *Silencer*® Negative Control siRNA (page 23).
2. Monitor siRNA transfection efficiency by transfecting a validated siRNA targeting an easy to assay target, then assaying target mRNA and/or protein levels.	Use a *Silencer* Positive Control siRNA such as *Silencer* GAPDH siRNA (#AM4624; page 23) to verify delivery efficiency and induction of RNAi. Assess effects with KDalert™ GAPDH Assay Kit (page 24), with GAPDH antibody (page 26), or by real-time PCR.
3. Improve confidence in RNAi data and control for sequence specific off-target effects by individually transfecting at least two different siRNAs in triplicate to the same target and comparing results.	Use *Silencer* Pre-designed siRNAs (page 16). Ambion guarantees that when you purchase 3 of these siRNAs to the same target, at least 2 will reduce mRNA levels by ≥70%.
4. Use siRNAs at their lowest effective levels, as higher siRNA concentrations lead to more off-target effects.	Use *Silencer* Pre-designed and/or Validated siRNAs (pages 16–17) at low concentrations; these siRNAs are designed for maximum potency and are typically effective at ≤10 nM.
5. Monitor both mRNA and protein levels to determine that the siRNA is working as intended, and to determine the proper timing of subsequent assays.	Isolate protein and RNA from the same sample with the PARIS™ Kit (page 39); monitor mRNA by real-time PCR using TaqMan® Gene Expression Assay (page 40) or other method and protein by Western blotting, immunofluorescence, or other techniques.

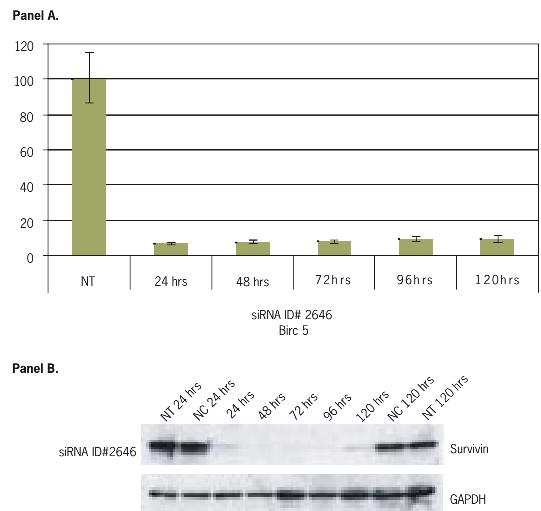


Figure 8. Timecourse of Survivin siRNA Effects on Survivin (BIRC5) mRNA and Protein Levels. HeLa cells were transfected with 30 nM *Silencer*® Pre-designed siRNA (ID: 2646) or *Silencer* Negative Control #1 siRNA in triplicate. **Panel A:** mRNA levels were monitored by real-time PCR using the corresponding TaqMan® Gene Expression Assay after isolation of RNA with the MagMAX™ Total RNA Isolation Kit and subsequent conversion to cDNA. Data from the survivin siRNA transfected cells are plotted relative to negative control siRNA transfected cells. **Panel B:** Survivin protein levels were analyzed by Western blot using the Western-Light™ Immunodetection System.

Elucidating Gene Function with RNAi Screens

RNAi screening, which involves systematically knocking down the expression of a set of genes, is an ideal tool to identify genes with roles in specific cellular functions. The rapid, reverse genetics process involves high-throughput screening to identify siRNAs (or dsRNAs) that induce an interesting phenotype. The entire screening process includes five steps:

1. Design Experiment and choose an appropriate siRNA (or dsRNA) library

RNAi screens require a set, or "library", of siRNAs (or dsRNAs for *C. elegans* and *Drosophila* screens) to a group of genes. The group of genes targeted can be large—for instance all known genes in the genome—or can be as small as 20 genes or less. With siRNA libraries, most researchers use multiple individual siRNAs per target in their screens to enhance confidence in the data. *Silencer*® siRNA Libraries, which generally include 3 siRNAs per target, are available for human and mouse genomes, the "druggable" genome, and for individual gene classes such as kinases, nuclear receptors, and the like. Custom siRNA libraries, in which you define the set of genes you wish to target and the number of siRNAs per target, are also available; see page 20–21 for details.

Among the most critical aspects of experimental design for siRNA screening are selection of siRNAs to screen and to use as controls, and the cell type(s) in which the experiment is to be performed. These decisions should be based on the biological pathway under study.

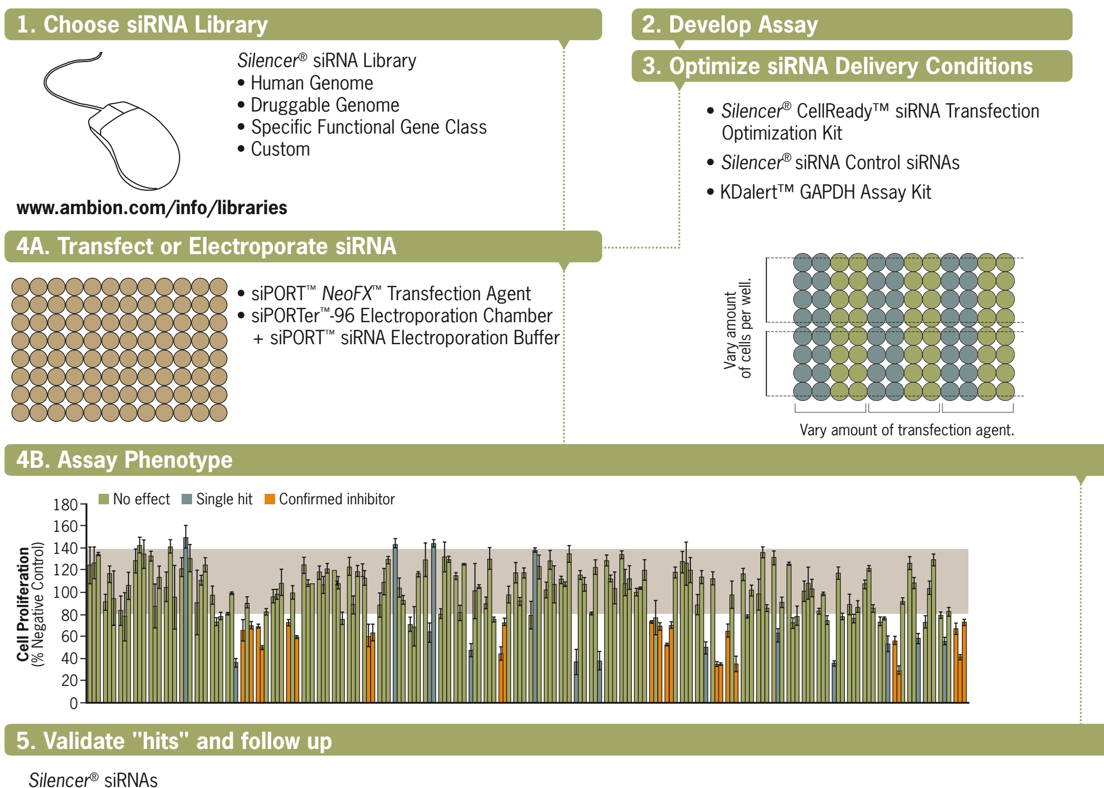
2. Develop a quantitative assay to monitor the cellular process being studied

Assays that measure the intensity of a cellular phenotype range from microscopic assays that monitor cell size and morphology, cell cycle status, or antibody staining, to enzymatic assays that assess the turnover of a specific substrate in a cell lysate, to direct measurements of biomolecules or small molecules in lysates, on cells, or in culture medium. In genome-scale screens, results are typically analyzed using plate readers or high-throughput imaging systems—the choice depends on the biological endpoint being assayed.

3. Optimize high-throughput transfection conditions for the desired cells

Efficient, reproducible siRNA delivery is essential for effective siRNA library screens. Because it is so critical, we highly recommend investing the time to select the best transfection method and transfection conditions for the cells that are to be used for siRNA library screening. Careful optimization of procedures will limit transfection variability and enhance the quality of screening results.

Figure 9. Steps Required for RNAi Screening in Mammalian Cell Systems



There are two basic methods employed for siRNA delivery—lipid mediated transfection and electroporation. Both are adaptable to medium and high throughput siRNA delivery, and methods for optimizing siRNA delivery are described on pages 10–11.

4. Perform screen and analyze data

With a robust phenotypic assay and optimized transfection conditions established, a library of siRNAs can be introduced sequentially into cells. Triplicate transfections for each siRNA provide enough data for reasonable statistical analysis. Automated liquid handling systems can reduce labor, but are not required; we often perform small scale screens with multichannel pipettors. Positive and negative control siRNAs on each plate provide quantitative numbers so that data from different plates can be normalized, and they provide the range of phenotypes needed to identify siRNAs that rate as "hits" (results outside the range of negative control samples that warrant further follow up).

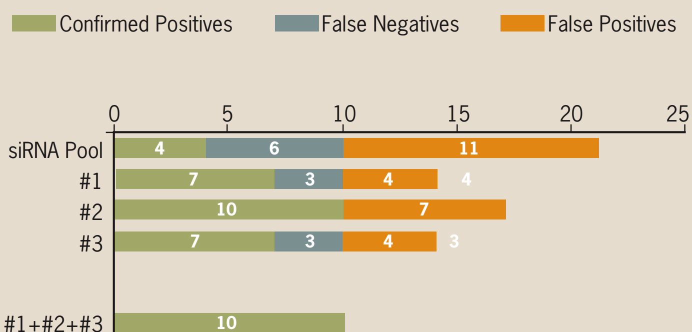
5. Validate hits and follow up

Validating a candidate hit involves a second or third screening pass and ultimately showing that the observed phenotype is specifically due to reducing the expression of the target gene. We confirm a hit by individually transfecting three or more different siRNAs targeting the same gene, monitoring reduction in the target mRNA and/or protein, and then measuring the phenotype by one or more assays. In theory, all siRNAs that similarly reduce the expression of a target gene should yield the same phenotype. In practice, we find that occasional off-target effects of siRNAs lead to false positive or false negative phenotypes for a given siRNA. A major goal of the second screening pass is to retest all candidate genes to identify interesting hits and eliminate false positives. These validation experiments also serve to further refine the relevance of candidate hits with respect to the biological process of interest.

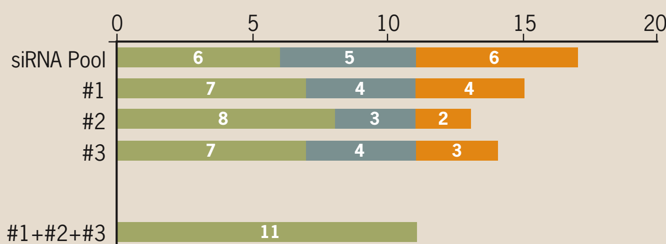
More information about RNAi screening can be found on the Ambion website at **www.ambion.com/RNAi**. Ambion has also recently published a 130-page guide to siRNA screening. To receive your free copy, contact us or visit the web at **www.ambion.com/RNAi/screening**.

Individual siRNAs Enhance Reliability

Although screening with siRNA pools minimizes screening costs, screening with three individual siRNAs per gene significantly decreases false positive and false negative rates, resulting in enhanced confidence in the data, reduction in the chance that important genes will be missed, and reduction in the amount of time spent following up on false positive "hits" from the screen. Ambion provides siRNAs in its libraries individually and/or as siRNA pools.



Panel A.



Panel B.

Figure 10. Individual siRNAs Outperform siRNA Pools in RNAi Screening. Three different siRNAs (#1, #2, and #3) to each of 59 different human kinase targets were transfected both individually and as pools in two different experiments. In one experiment, cell proliferation was monitored 48 hours after transfection. In the other experiment, apoptosis was induced by etoposide 24 hours post-transfection and caspase 3 activity was monitored 48 hours later. Confirmed "hits" were identified, where two of the three individual siRNAs resulted in significantly lower cell number or caspase 3 activity than negative control samples. The siRNA pools resulted in false positive and false negative rates that were no better than using a single, individual siRNA. Best results were obtained when three siRNAs to a single target were used in independent transfections (1+2+3).

Silencer® Pre-designed siRNAs

- Guaranteed silencing
- Designed for maximum potency and specificity
- Available against all human, mouse, and rat genes listed in RefSeq
- Searchable database makes finding siRNAs easy
- Provided with free sequence information

Application:
For inducing transient RNAi in cultured human, mouse, and rat cells

Delivery Method:
siRNAs can be delivered into cultured cells by transfection or electroporation

Silencer® siRNAs Are Remarkably Effective and Guaranteed to Silence

Silencer Pre-designed siRNAs—chemically synthesized, ready-to-use siRNAs available for all human, mouse, and rat genes—are designed with one of the most rigorously tested siRNA design algorithms in the industry (Figure 11; also see page 8 for more details). Importantly, Ambion guarantees that when three *Silencer* Pre-designed siRNAs are obtained to the same target, at least two will reduce target mRNA levels by 70% or more. In addition, because their design has been meticulously optimized, *Silencer* siRNAs effectively induce RNAi at low siRNA concentrations (Figure 12). Using carefully designed siRNAs at low concentrations minimizes off-target effects and maximizes gene silencing success.

High Quality Synthesis

Ambion synthesizes and purifies each siRNA in state-of-the-art facilities to meet the highest quality standards. As part of our rigorous quality control procedures, Ambion analyzes the mass of each RNA oligonucleotide by MALDI-TOF mass spectrometry and assesses the purity of all HPLC and PAGE purified oligonucleotides by HPLC. Finally, Ambion analyzes each annealed siRNA by gel electrophoresis to confirm that the strands annealed properly. The result is premium quality siRNA that is purified and ready to use. See page 8 for a description of siRNA purity grades.

Silencer siRNAs Are Easy to Obtain

Ambion's searchable online siRNA database makes it easy to obtain effective, guaranteed-to-work siRNAs. Simply visit **www.ambion.com/siRNA** to find siRNAs for your human, mouse, or rat gene of interest. Interested in other organisms? Ambion provides a custom design service (see page 18). More details can be found on pages 8–9.

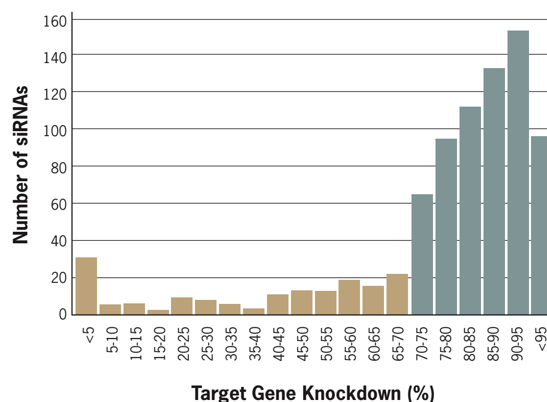


Figure 11. Effectiveness of *Silencer*® Pre-designed siRNAs. This graph shows the distribution of gene silencing measured for 808 *Silencer* Pre-designed siRNAs targeting >300 endogenously expressed human genes. Target mRNA levels were measured by qRT-PCR 48 hr after transfection into HeLa cells. More than 82% of the siRNAs successfully silenced their targets, which means that Ambion can guarantee *Silencer* siRNAs will be effective. Data courtesy of Cenix BioScience.

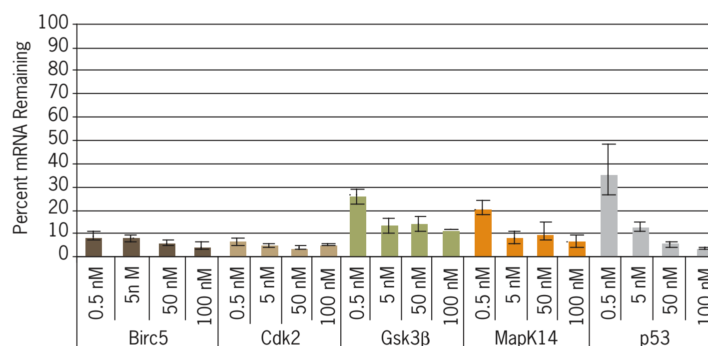


Figure 12. Potency of *Silencer*® siRNAs at Low Concentrations. siRNAs were transfected into HeLa cells at the indicated concentrations, and target mRNA levels were monitored by real-time RT-PCR 48 hr after transfection using the appropriate TaqMan® Gene Expression Assay. Transfections were performed in triplicate, and the data are shown relative to *Silencer* Negative Control #1 transfected cells.

ORDERING INFORMATION

Cat#	Description	Size
AM16708	*Silencer*® Pre-designed siRNA, standard purity, annealed	5 nmol
AM16708A	*Silencer*® Pre-designed siRNA, standard purity, annealed – each siRNA when 3 or more purchased to the same target	5 nmol
AM16704	*Silencer*® Pre-designed siRNA, standard purity, annealed	20 nmol
AM16706	*Silencer*® Pre-designed siRNA, standard purity, annealed	40 nmol
AM16810	*Silencer*® Pre-designed siRNA, HPLC purified, annealed	20 nmol
AM16804	*Silencer*® Pre-designed siRNA, HPLC purified, annealed	40 nmol
AM16806	*Silencer*® Pre-designed siRNA, HPLC purified, annealed	160 nmol
AM16904	*Silencer*® Pre-designed siRNA, PAGE purified, annealed	25 nmol
AM16830	*Silencer*® Pre-designed siRNA, In Vivo Ready	100 nmol
AM16831	*Silencer*® Pre-designed siRNA, In Vivo Ready	250 nmol
AM16832	*Silencer*® Pre-designed siRNA, In Vivo Ready	1 µmol
AM16833	*Silencer*® Pre-designed siRNA, In Vivo Ready	10 µmol
AM16811	*Silencer*® Pre-designed siRNA, Cy™3 labeled	20 nmol
AM16812	*Silencer*® Pre-designed siRNA, FAM™ labeled	20 nmol

See pages 8-9 for ordering instructions and information on purity options.

For Research Use Only. Not for use in diagnostic procedures.

Silencer Pre-designed siRNAs are available for essentially all genes in the human, mouse, and rat genomes; see **www.ambion.com/siRNA** for details.

Silencer® Validated siRNAs

Verified and Guaranteed to Induce Silencing

- Functionally proven and guaranteed to reduce target mRNA levels 70% or more
- Designed using the same algorithm as *Silencer*® Pre-designed siRNAs
- Available for hundreds of highly studied human genes
- Supplied ready to use with silencing data and sequence information

Application:
For inducing transient RNAi in cultured human cells

Delivery Method:
siRNAs can be delivered into cultured cells by transfection or electroporation

Silencer Validated siRNAs are individual siRNA duplexes that have already been verified experimentally to reduce the expression of their individual target genes. Each siRNA was designed using the same effective algorithm used to design Ambion's *Silencer* Pre-designed siRNAs (page 16). However, each one has also been functionally proven to and is guaranteed to reduce target mRNA levels by at least 70% 48 hours post transfection.

All validated siRNAs are annealed and provided ready to use. The data sheet accompanying each siRNA indicates the extent of mRNA knockdown observed, the exon targeted by the siRNA, and full siRNA sequence information. See page 8 for a description of siRNA purity grades.

	Silencer® Pre-designed siRNAs	*Silencer*® Validated siRNAs
Guarantee	At least 2 guaranteed to reduce target mRNA levels by 70% or more when 3 obtained to the same target	Each one guaranteed to reduce target mRNA levels by 70% or more
Targets	Available for all human, mouse, and rat genes	Available for hundreds of human genes
Validation status	Not validated	Experimentally validated and provided with silencing data
Sequence information	Provided free	Provided free

Table 1. Comparison of *Silencer*® Pre-designed and Validated siRNAs

To find *Silencer* Pre-designed and Validated siRNAs, visit **www.ambion.com/siRNA**.

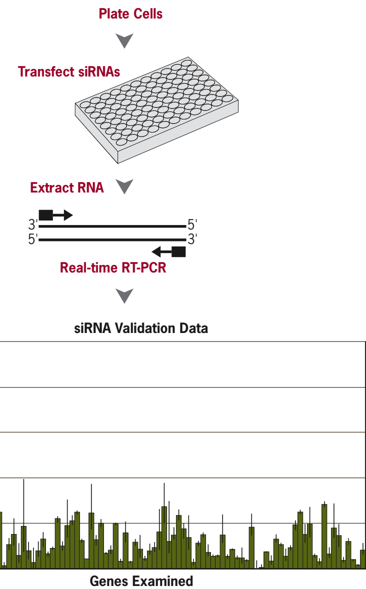


Figure 13. Validation of Ambion's *Silencer*® Validated siRNAs. The following procedure is used to validate siRNAs:

1. Gene specific and negative control siRNAs are independently transfected in triplicate.
2. 48 hours later, RNA is extracted.
3. Target mRNA levels are quantitated by real-time PCR.
4. Data are normalized using 18S rRNA levels.
5. The extent of target gene knockdown is expressed as a percent of mRNA remaining in cells treated with the gene-specific siRNA compared to cells treated with a negative control siRNA (*Silencer*® Negative Control siRNA #1). Data courtesy of Cenix Bioscience.

ORDERING INFORMATION		
Cat#	Description	Size
AM51321	*Silencer*® Validated siRNA, HPLC Purity	5 nmol
AM51323	*Silencer*® Validated siRNA, HPLC Purity	20 nmol
AM51324	*Silencer*® Validated siRNA, HPLC Purity	40 nmol
AM51325	*Silencer*® Validated siRNA, HPLC Purity	160 nmol
AM51326	*Silencer*® Validated siRNA, Cy™3 Dye labeled	20 nmol
AM51326	*Silencer*® Validated siRNA, FAM™ Dye labeled	20 nmol
AM51331	*Silencer*® Validated siRNA, Std Purity	5 nmol
AM51333	*Silencer*® Validated siRNA, Std Purity	20 nmol
AM51334	*Silencer*® Validated siRNA, Std Purity	40 nmol
AM51340	*Silencer*® Validated siRNA, In Vivo Ready	100 nmol
AM51341	*Silencer*® Validated siRNA, In Vivo Ready	250 nmol
AM51342	*Silencer*® Validated siRNA, In Vivo Ready	1 µmol
AM51343	*Silencer*® Validated siRNA, In Vivo Ready	10 µmol

See pages 8-9 for ordering instructions and information on purity options.

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Custom siRNA Design

Let Us Take the Guesswork Out of siRNA Design

- Custom siRNA designs for any target, including individual splice variants
- Design algorithm results in highly effective siRNAs
- Obtain custom designed, chemically synthesized siRNA, or just sequence information

Application:
To obtain expert-designed siRNAs to silence genes not targeted by Ambion's *Silencer*® siRNAs.

Synthesis Method:
Ambion can chemically synthesize custom designed siRNAs, or can provide just the siRNA sequence information.

The siRNA design algorithm used by Ambion yields an extremely high percentage of effective siRNA sequences (see *siRNA Design: Ambion Answers Your Questions*, page 7). Multiple siRNAs are immediately available for human, mouse, and rat genes listed in the public RefSeq database maintained by NCBI; these siRNAs are known as *Silencer* Pre-designed siRNAs (page 16). siRNAs for other genes are available on a custom basis. If desired, you can purchase just the sequence information.

ORDERING INFORMATION

Cat#	Description	Size
AM16750	Custom siRNA Design Fee	per gene

For custom siRNA design for single splice variants or for organisms other than human, mouse, or rat (for siRNAs to human, mouse or rat genes, see page 16). Order custom siRNA design and the appropriate size and purity of *Silencer* Pre-designed siRNA (page 16) to obtain algorithm designed, chemically synthesized siRNAs.

Cat#	Description	Size
AM16751	*Silencer*® Pre-designed siRNA sequence information only	per siRNA sequence

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For more information, including how to order, see **www.ambion.com/siRNA.**

Custom siRNA Synthesis

High Quality siRNA Oligonucleotides: You Provide the Sequence, We'll Do the Rest

- Premium quality synthesis and purification
- Your choice of purification method, amount and format
- Modifications available
- Rigorous quality control

Application:
For inducing transient RNAi in cultured cells. Larger amounts of both unmodified and modified siRNAs can be used in animals.

Delivery Method:
siRNAs can be delivered into cultured cells by transfection or electroporation and into animals by injection or inhalation.

When you need high quality chemically synthesized siRNA, but prefer to provide your own siRNA sequence, you can rely on Ambion's custom siRNA synthesis service. High quality is guaranteed. All RNA oligonucleotides synthesized by Ambion are assessed by MALDI-TOF (matrix-assisted laser desorption ionization–time-of-flight) mass spectrometry to confirm siRNA identity and quality. In addition, each HPLC and PAGE purified siRNA strand is assessed for purity by analytical HPLC. Finally, all annealed siRNAs are analyzed by nondenaturing gel electrophoresis to confirm that the strands annealed properly. The result—premium quality siRNA you can trust.

Below is a partial list of custom siRNA synthesis options. See page 8 for a description of purity grades. For more information, see **www.ambion.com/siRNA** or contact us at **custom_services@ambion.com**.

ORDERING INFORMATION

Cat#	Description	Size
AM16104	Custom siRNA, standard purity	20 nmol
AM16106	Custom siRNA, standard purity	40 nmol
AM16204	Custom siRNA, HPLC purified	40 nmol
AM16206	Custom siRNA, HPLC purified	160 nmol
AM16210	Custom siRNA, HPLC purified	20 nmol
AM16304	Custom siRNA, PAGE purified	25 nmol
AM16230	Custom siRNA, In Vivo Ready	100 nmol
AM16231	Custom siRNA, In Vivo Ready	250 nmol
AM16232	Custom siRNA, In Vivo Ready	1 µmol
AM16233	Custom siRNA, In Vivo Ready	10 µmol
AM16211	Custom siRNA, Cy™3 Dye labeled	20 nmol
AM16212	Custom siRNA, FAM™ Dye labeled	20 nmol
AM16304	PAGE purified, annealed	25 nmol

See pages 8-9 for ordering instructions and information on purity options.

For Research Use Only. Not for use in diagnostic procedures.

Silencer® In Vivo Ready siRNAs

Ready for formulation or direct injection into animals

- Provided in convenient 100, 250, 1000 and 10,000 nmol sizes
- HPLC purified, dialyzed to remove salt, sterile filtered, and endotoxin tested
- Quality you can trust

Application:
For silencing genes in rodents and other animal models

Delivery Method:
Includes injection, inhalation, or infusion depending on targeted organ. See the In Vivo siRNA Resource at **www.ambion.com/siRNA/invivo**

For siRNA induced gene silencing experiments in animals, high quality siRNA is critical. Ambion's *Silencer*® In Vivo Ready siRNAs—the highest quality siRNAs available anywhere—provide the extra level of purification and testing required for introduction into animals. These siRNAs, once resuspended in the appropriate buffer or formulated as you desire, are ready for injection into animals.

Each siRNA strand is chemically synthesized, deprotected, and HPLC purified by ion exchange chromatography to produce >95% full length product. The purity of each siRNA strand is verified by analytical HPLC, and the mass is confirmed by MALDI-TOF mass spectrometry prior to annealing. After annealing, each double stranded siRNA molecule is analyzed by PAGE to verify annealing efficiency. Then, in preparation for in vivo use, each siRNA is dialyzed to remove salt, resulting in <1 microsiemens conductance in a 50 µM solution. Finally, the siRNAs are sterile filtered and endotoxin tested to guarantee their suitability for delivery into animals.

Silencer In Vivo Ready siRNAs are synthesized from a sequence you provide or from one of Ambion's *Silencer* Pre-designed or Validated siRNA sequences.

Silencing of GAPDH in Mouse Lung with *Silencer*® In Vivo Ready siRNA

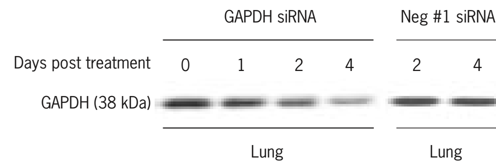

Figure 14. Silencing of GAPDH in Mouse Lung with *Silencer*® In Vivo Ready siRNAs. *Silencer* In Vivo Ready siRNAs were administered to mice intranasally after mixing with an amine-based delivery agent. Eight nmol GAPDH siRNA (4.3 mg/kg) or 16 nmol *Silencer* Negative Control #1 siRNA (8.6 mg/kg) were delivered per mouse. No toxicity was noted in either treatment group. GAPDH protein levels were reduced approximately 50% in the lungs of GAPDH siRNA treated mice after four days. Data courtesy of Dr. Sailen Barik, University of South Alabama.

ORDERING INFORMATION

Cat#	Description	Size
AM16230	Custom siRNA, In Vivo Ready	100 nmol
AM16231	Custom siRNA, In Vivo Ready	250 nmol
AM16232	Custom siRNA, In Vivo Ready	1 µmol
AM16233	Custom siRNA, In Vivo Ready	10 µmol
AM16830	Silencer® Pre-designed siRNA, In Vivo Ready	100 nmol
AM16831	Silencer® Pre-designed siRNA, In Vivo Ready	250 nmol
AM16832	Silencer® Pre-designed siRNA, In Vivo Ready	1 µmol
AM16833	Silencer® Pre-designed siRNA, In Vivo Ready	10 µmol
AM51340	Silencer® Validated siRNA, In Vivo Ready	100 nmol
AM51341	Silencer® Validated siRNA, In Vivo Ready	250 nmol
AM51342	Silencer® Validated siRNA, In Vivo Ready	1 µmol
AM51343	Silencer® Validated siRNA, In Vivo Ready	10 µmol

See pages 8-9 for ordering instructions and information on purity options.

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In Vivo siRNA Resource

References, protocols, and delivery information on the use of siRNAs in animals are available at **www.ambion.com/siRNA/invivo**

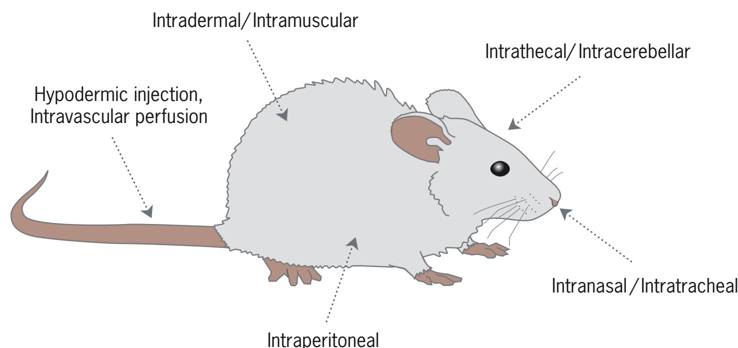

Figure 15. Strategies for Delivery of siRNA Molecules In Vivo.

Silencer® siRNA Libraries

For RNAi screening projects, large and small

- Effective—siRNAs meticulously designed for maximum potency and specificity
- Convenient—Ready-to-use siRNAs in amounts sufficient for hundreds of transfections
- Reliable—Three individual siRNAs per target increase screening accuracy
- Comprehensive—Available for small gene sets and for the whole human genome
- Customizable—Custom aliquots, pooling, and custom libraries available

Expedite Gene Function Analysis with Libraries of Effective siRNAs

Increasingly, researchers are harnessing the power of RNAi to better understand gene function. To support this growing demand, Ambion provides pre-defined and custom sets of premium quality siRNAs—known as *Silencer*® siRNA Libraries—for performing RNAi experiments in human, mouse, and rat cell systems.

siRNA Designs That Maximize Specific Silencing

Silencer siRNA Libraries feature combinations of *Silencer* Pre-designed siRNAs (page 16), and where available, *Silencer* Validated siRNAs (page 17) provided in 96-well or 384-well plates. The design of each siRNA has been carefully optimized to provide potent and specific silencing. Indeed, the siRNA selection process yields siRNAs that are effective at low concentrations (Figure 12, page 16), enabling screening experiments at 30 nM siRNA or lower, which minimizes reagent costs, as well as minimizes off-target effects.

To ensure specificity, the siRNA design algorithm incorporates a rigorous check of all siRNA sequences against the transcriptome of the organism targeted. This process is accomplished via a modified alignment search tool, which effectively identifies short regions of sequence alignment that BLAST searches miss. Sequences that have significant regions of sequence similarity to off-target transcripts are eliminated. Also eliminated are sequences that fall in polymorphic regions (e.g., SNPs) and sequences predicted to invoke an antiviral response. In addition, the siRNA design process incorporates stringent rules to prevent off-strand targeting.

Pre-plated siRNA Libraries for Human Kinases, Phosphatases, and the Druggable Human Genome

Ambion provides its most popular *Silencer* siRNA Libraries in a convenient, ready-to-ship format. These libraries feature three individual siRNAs per target, each at 0.25 nmol, which is sufficient for 100 transfections at 25 nM in 96 well plates. Screening with three individual siRNAs per gene, as compared to screening with fewer individual siRNAs or with pools of siRNAs, significantly decreases both false positive and false negative rates.This results in enhanced confidence in RNAi screening data, reduction in the chance that important genes will be missed, and reduction in the amount of time spent following up on false positive "hits" from the screen. However, we realize that siRNA pools provide a cost advantage when performing expensive downstream assays. For this reason, siRNAs in the pre-plated *Silencer* siRNA Libraries are arranged in 96 well plates such that siRNAs to the same target are on different plates in the same well location, making it easy to pool the siRNAs if desired. An empty column on each plate is provided so that you can readily add assay-specific controls to daughter plates without the time and effort required to re-array the siRNAs in the plates.

Human Druggable Genome siRNA Library

The *Silencer* Human Druggable Genome siRNA Library V3 is a popular choice for many researchers. Conveniently arranged such that siRNAs are grouped by gene functional class, this library targets the most therapeutically relevant genes in the human genome (Figure 16). The gene classes and number of genes in each class targeted by the *Silencer* Druggable Genome siRNA Library are outlined in Table 3. As for all Ambion *Silencer* siRNA Libraries, the list of genes targeted by this library is available upon request. Simply email us at **libraries@ambion.com** to request this information.

Human Kinase siRNA Library

Because of their importance in cell signaling and many biological pathways, kinases are key drug targets and the subjects of intense scrutiny. The *Silencer* Human Kinase siRNA Library V3 targets 719 human kinases with three individual siRNAs per gene. This siRNA library enables systematic, yet cost effective, studies of these key cell regulators by RNAi. More than 775 siRNAs included in the *Silencer* Human Kinase siRNA Library V3 have been bench tested and validated to knockdown target mRNA levels by 70% or more, imparting enhanced confidence in silencing results.

Human Phosphatase siRNA Library

Along with kinases, phosphatases are key regulators of biological pathways and cell signaling cascades. The *Silencer* Human Phosphatase siRNA Library V3 targets 267 human phosphatases with three individual siRNAs per gene. This library is the ideal companion to the *Silencer* Kinase siRNA Library V3, enabling more detailed studies of key biological regulators.

Pre-defined siRNA Libraries Plated to Order

Pre-defined, made-to-order *Silencer* siRNA Libraries are available for the human genome, druggable human genome, and dozens of gene functional classes. Mouse and rat siRNA libraries are also available in this format. To create these siRNA libraries, the Ambion bioinformatics team uses PANTHER™ (**www.pantherdb.org**) and Gene Ontology™ annotation information to create up-to-date target gene sets. siRNAs targeting the collection are then plated to order (minimum 1 nmol), giving you not only the most up-to-date siRNA libraries, but also the freedom to customize your library so that it is most convenient for your particular experimental setup. Want to add siRNAs to your collection? No problem! Ambion can also provide siRNAs in 96-well or 384 well plates, deliver multiple aliquots of each plate, arrange siRNA in custom configurations, and provide siRNAs individually and/or in pools.

Silencer® siRNA Libraries, cont.

Silencer® Human Druggable Genome (V3) & *Silencer*® Human Extended Druggable Genome (V3) siRNA Libraries			
Functional Categories	**Gene Count**	**Functional Categories**	**Gene Count**
Adhesion Molecule	156	Polymerase	43
Carrier-related Protein	176	Protease	449
Cytoskeletal Protein	426	Protease Inhibitor	102
Deacetylase	24	Receptor	512
Dehydratase-related	31	Reductase	135
Dehydrogenase-related	194	Signaling Molecule	444
Esterase-related	43	Synthase	95
G Protein	184	Synthetase	69
G Protein-Coupled Receptor	334	Transferase	669
Hydrolase	500	Transporter	471
Hydroxylase	16	Tubulin	21
Ion Channel	307	**Total (*Silencer*® Druggable Genome)**	**7784**
Isomerase	119	Cell Junction Protein	185
Kinase	665	Chaperone	115
Kinase-related	101	Defense/Immunity Response	224
Ligase	307	Extracellular Matrix	178
Lipase	57	Hydratase	20
Lyase	81	Membrane Traffic Protein	141
Miscellaneous	673	Ribosomal Protein	54
Nuclear Hormone Receptor	32	Select Calcium Binding Proteins	209
Nucleic Acid Binding	1139	Select Regulatory Molecule	36
Oxidase	81	Synthase/Synthetase	8
Oxidoreductase	57	Transcription Cofactor	101
Oxygenase	93	Transcription Factor	1227
Phosphatase	231	Translation-related	42
Phosphodiesterase	35	**Total (*Silencer*® Extended Druggable Genome)**	**9102**

Table 3. *Silencer*® Human Druggable Genome siRNA Library V3

Custom siRNA Libraries—The Ultimate in Flexibility

Do you have a favorite list of genes you would like to target with a collection of siRNAs? Ambion can prepare a custom *Silencer* siRNA Library to any set of human, mouse, or rat genes. The minimum order is only 50 siRNAs. All you need to supply is a list of genes or transcripts identified by NCBI Entrez Gene ID or RefSeq mRNA accession number. With this user-friendly option, you can specify the order of the siRNAs on the plate, as well as the amount of siRNA per order (1 nmol minimum), number of aliquots of each plate and the number of siRNAs per target. siRNAs can be provided individually or pooled.

Information to Accelerate Discovery

All *Silencer* siRNA Libraries, whether custom, pre-defined, or pre-plated, are supplied with full siRNA sequence information, and when available, with siRNA validation data. Also provided on the CD accompanying the siRNA library are gene annotation information, which includes gene symbol, gene name, aliases, NCBI Entrez Gene ID, associated RefSeq mRNA accession numbers, and other accession numbers. Plates are individually barcoded with unique identifiers; this information is also provided with the siRNA and gene information.

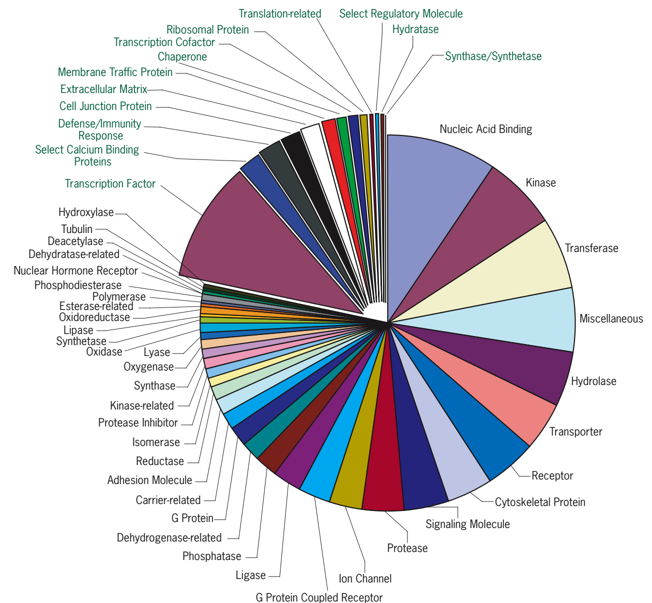

Figure 16. Number of Genes Targeted by the *Silencer*® Human Druggable Genome siRNA Libraries and Their Available siRNA Library Subsets. The *Silencer* Human Druggable Genome siRNA Library V3 targets 7784 therapeutically relevant genes, whereas the *Silencer* Human Extended Druggable Genome siRNA Library V3 targets 9102 human genes. siRNA libraries to all of the subsets listed above are also available as pre-defined, plated-to-order siRNA libraries.

ORDERING INFORMATION

For pricing and additional information, see **www.ambion.com/info/libraries** or contact us by phone (see back cover) or via email (**libraries@ambion.com**).

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Silencer® CellReady™ siRNA Transfection Optimization Kit

- Demystifies and accelerates transfection optimization
- Includes 96 well plates of control siRNAs and siPORT™ *NeoFX*™ Transfection Agent
- Convenient and fast—simply add transfection agent, add cells, incubate, and assay
- Efficiency of GAPDH siRNA delivery readily monitored by real-time PCR or the KDalert™ GAPDH Assay Kit

Application:
For determining optimal transfection conditions of human, mouse, and rat cells in a 96 well format

Delivery Method:
Formatted for 96 well transfection; reverse transfection recommended

Determining the ideal siRNA transfection parameters for a particular cell type can be a daunting task. Developed specifically to accelerate siRNA transfection optimization in 96 well plates, the *Silencer*® CellReady™ siRNA Transfection Optimization Kit provides pre-plated siRNA controls, siPORT *NeoFX* Transfection Agent, and a detailed Instruction Manual that leads you step by step through this process, reducing the time needed for complete transfection optimization. Since the reagents are provided in a ready-to-use format, and the procedure is straightforward and described in detail, you will not be tempted to skip the optimization of critical parameters. The result: final transfection conditions that improve siRNA delivery.

Simple to Use, Easy to Assay Controls

At the heart of the *Silencer* CellReady siRNA Transfection Optimization Kit are three 96 well plates containing multiple aliquots of a validated positive control siRNA that targets human, mouse, and rat GAPDH, as well as *Silencer* Negative Control #1 siRNA, a non-targeting, negative control. These two siRNAs, each aliquotted into 48 wells of the 96 well plates (Figure 17A), are pre-plated and ready for transfection.

To use, simply add the desired amount of transfection agent (0.4 mL siPORT *NeoFX* siRNA Transfection Agent is supplied, but other transfection agents can also be used), briefly incubate, and then overlay with the desired number of cells. Since the positive control siRNA targets GAPDH, a well-studied housekeeping gene expressed in all human, mouse, and rat cell types and often used as a control, target knockdown can be readily monitored using commonly available assays. siRNA induced knockdown of GAPDH protein can also be quickly and easily monitored with the new KDalert GAPDH Assay Kit, which assesses GAPDH enzymatic activity (see page 24). The convenient arrangement of siRNAs on each plate (Figure 17A) can be used to assess the effectiveness of up to 24 different transfection conditions in duplicate. The Instruction Manual includes a plate map and step by step procedures for setting up optimization experiments.

ORDERING INFORMATION

Cat#	Description	Size
AM86050	*Silencer*® CellReady™ siRNA Transfection Optimization Kit	3 plates+0.4 mL transfection agent

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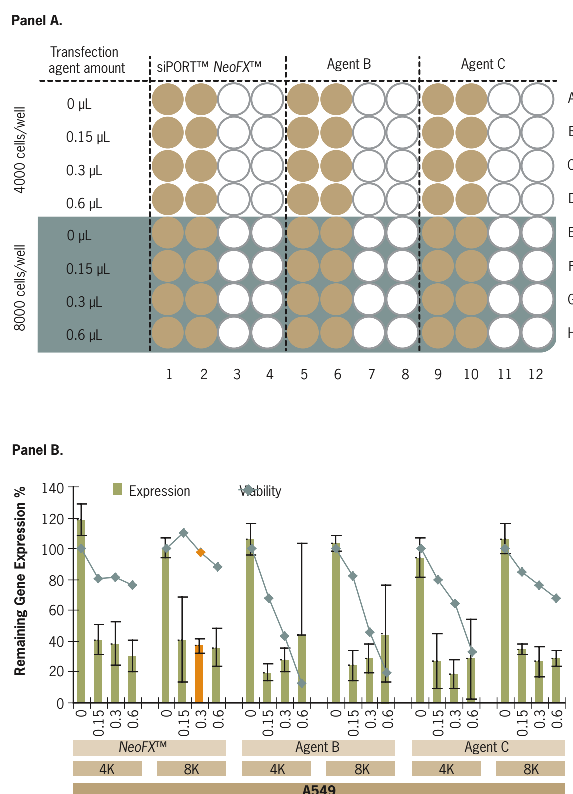

Figure 17. Optimization of siRNA Transfection Using the *Silencer*® CellReady™ siRNA Transfection Optimization Kit. Panel A. Experimental design of a transfection optimization experiment using the *Silencer* CellReady siRNA Transfection Optimization Kit. **Panel B.** Cell viability and knockdown of GAPDH levels in A549 cells was measured using the KDalert™ GAPDH Assay Kit 48 hours after the experiment was performed as described in A. The bar marked in orange represents the conditions eliciting efficient knockdown while maintaining high cell viability.

Silencer® Negative Control siRNAs

- Non-targeting siRNAs that have limited sequence similarity to known genes
- Validated for use in human, mouse, and rat cells
- Functionally proven to have minimal effects on cell proliferation and viability

Application:
Controls for transient RNAi experiments in cultured human, mouse, and rat cells

Delivery Method:
siRNAs can be delivered into cultured cells by transfection or electroporation

Negative Controls

Negative control siRNAs—siRNAs with sequences that do not target any gene product—are essential for determining transfection efficiency and to control for the effects of siRNA delivery. Ambion has designed and tested seven Negative Control siRNAs that have no significant sequence similarity to mouse, rat, or human gene sequences. They have all been tested in cell-based screens and proven to have no significant effect on cell proliferation, viability, or morphology.

Silencer siRNA Screening Control Panel

Most researchers carefully test their negative control siRNAs before choosing one or more for inclusion in an siRNA library screen. The *Silencer*® siRNA Screening Control Panel, which includes all seven negative control siRNAs, simplifies this process. As an added benefit, a positive control siRNA to human, mouse, and rat KIF11 (Eg5) is included. Knockdown of KIF11, which encodes a kinesin family motor protein, leads to mitotic arrest, so effective delivery of KIF11 siRNA can be assessed visually or using a cell based assay.

Interested in larger amounts or In Vivo Ready formats of any Negative Control siRNA? Contact us at **custom_services@ambion.com** for a quote.

ORDERING INFORMATION

Cat#	Description	Size
AM4611	*Silencer*® Negative Control #1 siRNA	5 nmol (50 µM)
AM4635	*Silencer*® Negative Control #1 siRNA	40 nmol
AM4636	*Silencer*® Negative Control #1 siRNA	5 x 40 nmol
AM4620	*Silencer*® FAM™ Dye labeled Negative Control #1 siRNA	5 nmol
AM4621	*Silencer*® Cy™3 Dye labeled Negative Control #1 siRNA	5 nmol
AM4613	*Silencer*® Negative Control #2 siRNA	5 nmol (50 µM)
AM4637	*Silencer*® Negative Control #2 siRNA	40 nmol
AM4638	*Silencer*® Negative Control #2 siRNA	5 x 40 nmol
AM4615	*Silencer*® Negative Control #3 siRNA	5 nmol (50 µM)
AM4641	*Silencer*® Negative Control #4 siRNA	5 nmol (50 µM)
AM4642	*Silencer*® Negative Control #5 siRNA	5 nmol (50 µM)
AM4643	*Silencer*® Negative Control #6 siRNA	5 nmol (50 µM)
AM4644	*Silencer*® Negative Control #7 siRNA	5 nmol (50 µM)
AM4640	*Silencer*® siRNA Screening Control Panel (Includes negative control siRNAs #1–7, plus KIF11 siRNA)	8 x 1 nmol

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Silencer® Positive Control siRNAs

- Validated siRNA controls for optimizing siRNA experiments
- Most positive siRNA controls provided with non-targeting negative controls
- Functionally tested in several common cell lines

Application:
Controls for transient RNAi experiments in cultured human, mouse, and rat cells

Delivery Method:
siRNAs can be delivered into cultured cells by transfection or electroporation

Ambion's highly validated, positive siRNA controls serve multiple functions. First, because they are validated to work in multiple cell lines, they are ideal "test" siRNAs for those just beginning siRNA experiments. In addition, because they correspond to genes frequently used as internal controls, their effects are easy to assay, and thus provide an excellent tool to monitor siRNA transfection efficiency.

Each *Silencer*® Positive Control siRNA is validated for use in human cell lines. GAPDH, KIF11 (Eg5), GFP, and luciferase siRNAs are also validated in mouse and rat cells. The most versatile positive control siRNA is GAPDH siRNA for human, mouse, and rat. GAPDH is ubiquitously expressed, and this siRNA's effect can be measured by assays most researchers have on hand, as well as with the KDalert™ GAPDH Assay Kit (page 24).

Silencer Negative Control #1 siRNA (2 nmol) is included with the 5 nmol sizes of *Silencer* Positive Control siRNAs. For measuring silencing at the protein level, a selection of antibodies are available, including antibodies against GAPDH, ß-actin, and cyclophilin (see page 26).

ORDERING INFORMATION

Cat#	Description	Size
AM4624	*Silencer*® GAPDH siRNA (Human, Mouse, Rat)	5 nmol + 2 nmol Neg Control (50 µM)
AM4631	*Silencer*® GAPDH siRNA (Human, Mouse, Rat)	40 nmol
AM4632	*Silencer*® GAPDH siRNA (Human, Mouse, Rat)	5 x 40 nmol
AM4605	*Silencer*® GAPDH siRNA (Human)	5 nmol + 2 nmol Neg Control (50 µM)
AM4633	*Silencer*® GAPDH siRNA (Human)	40 nmol
AM4634	*Silencer*® GAPDH siRNA (Human)	5 x 40 nmol
AM4622	*Silencer*® FAM™ Dye labeled GAPDH siRNA (human)	5 nmol
AM4623	*Silencer*® Cy™3 Dye labeled GAPDH siRNA (human)	5 nmol
AM4607	*Silencer*® beta-actin siRNA	5 nmol + 2 nmol Neg Control (50 µM)
AM4616	*Silencer*® Cyclophilin siRNA	5 nmol + 2 nmol Neg Control (50 µM)
AM4619	*Silencer*® Lamin A/C siRNA	5 nmol + 2 nmol Neg Control (50 µM)
AM4639	*Silencer*® KIF11 (Eg5) siRNA (Human, Mouse, Rat)	5 nmol + 2 nmol Neg Control (50 µM)
AM4625	*Silencer*® GFP (Cycle 3) siRNA	5 nmol + 2 nmol Neg Control (50 µM)
AM4626	*Silencer*® GFP (eGFP) siRNA	5 nmol + 2 nmol Neg Control (50 µM)
AM4627	*Silencer*® Firefly Luciferase (GL2) siRNA	5 nmol + 2 nmol Neg Control (50 µM)
AM4628	*Silencer*® Firefly Luciferase (GL3) siRNA	5 nmol + 2 nmol Neg Control (50 µM)
AM4629	*Silencer*® Firefly Luciferase (GL2 + GL3) siRNA	5 nmol + 2 nmol Neg Control (50 µM)
AM4630	*Silencer*® Renilla Luciferase siRNA	5 nmol + 2 nmol Neg Control (50 µM)

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KDalert™ GAPDH Assay Kit

- Assess GAPDH siRNA delivery in less time and for a fraction of the cost of real-time PCR
- Analyze from one to 96 samples simultaneously
- Measure both GAPDH siRNA induced knockdown AND transfection induced toxicity
- Use with *Silencer®* GAPDH siRNA Controls and *Silencer* CellReady™ Transfection Optimization Kit for a complete transfection optimization solution

Application:
For assessing GAPDH enzymatic activity—use with GAPDH siRNA and Negative Control siRNA to optimize transfection parameters in human, mouse, and rat cells.

With the KDalert™ GAPDH Assay Kit, you can obtain a reliable measure of GAPDH enzyme activity in cultured human, mouse, or rat cells in less than 30 minutes using a microplate fluorometer or spectrophotometer. The KDalert GAPDH Assay Kit, which also measures transfection induced cytotoxicity, is ideal for use with Ambion's *Silencer* GAPDH siRNA. GAPDH is a ubiquitously and highly expressed gene, making this siRNA an ideal positive control for transfection optimization experiments in human, mouse, and rat cells.

Rapid, Time-saving Procedure

To use the KDalert GAPDH Assay Kit to optimize siRNA transfection, individual cell samples are transfected with GAPDH siRNA or negative control siRNA. Two to three days after transfection, the included cell lysis buffer is added to the cells, samples are incubated for 20 minutes, the diluted master mix of assay reagents is added, and then the level of fluorescence is read four minutes later in a microplate or standard fluorometer (Figure 18); a spectrophotometer can also be used to monitor a change in absorbance.

One Simple Assay for Two Critical Readouts

Once the assay is complete, siRNA delivery efficiency is assessed by comparing GAPDH activity levels in the GAPDH siRNA- and negative control siRNA-treated samples. The assay can also be used in the same experiment to monitor transfection agent induced toxicity. For this analysis, GAPDH activity from negative control siRNA transfected cells is compared to that of untreated cells. Reduced GAPDH activity in negative control transfected cells compared to non-transfected cells is an indication that the transfection induced cytotoxicity.

Part of Ambion's Complete Solution for siRNA Transfection and Transfection Optimization

The KDalert GAPDH Assay Kit integrates seamlessly with other Ambion products used for siRNA transfection and transfection optimization, such as: *Silencer* GAPDH Control siRNAs (see page 23), *Silencer* Negative Control siRNAs (see page 23), *Silencer* CellReady Transfection Optimization Kit (see page 22), and the *Silencer* siRNA Transfection II Kit (see page 30).

ORDERING INFORMATION		
Cat#	Description	Size
AM1639	KDalert™ GAPDH Assay Kit	375 rxns

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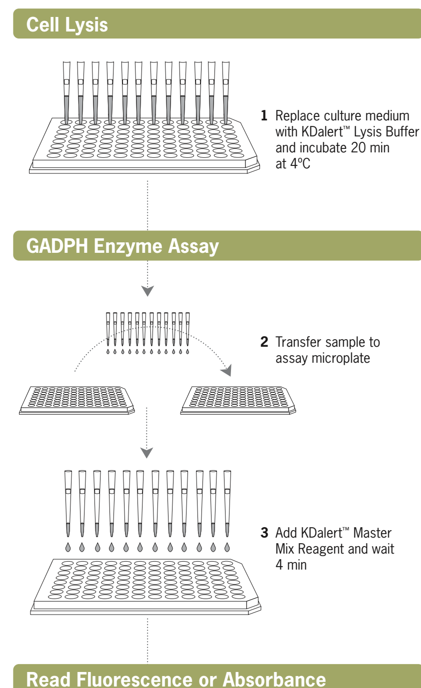

Figure 18. KDalert™ GAPDH Assay Kit Protocol.

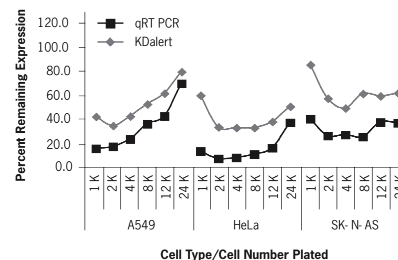

Figure 19. Comparison of GAPDH Enzyme Activity and qRT-PCR Data. The indicated number of cells from three different cell lines were transfected with *Silencer®* GAPDH siRNA and *Silencer* Negative Control #1 siRNA. The remaining GAPDH expression 48 hr after transfection for GAPDH siRNA-transfected cultures was calculated as a percentage of expression of Negative Control siRNA-transfected cultures. The KDalert™ GAPDH Assay was used to measure GAPDH enzyme activity, and qRT-PCR was used to measure GAPDH mRNA. The KDalert GAPDH Assay data mirrored that from qRT-PCR, indicating that it accurately detects siRNA induced knockdown of GAPDH.

Silencer® Labeled Control siRNAs

- Fluorescently labeled siRNAs simplify the monitoring of siRNA delivery
- Labeled GAPDH siRNA is ideal for correlating siRNA uptake with mRNA knockdown
- Functionally tested in several common cell lines
- Fluorescein derivative (FAM™) and Cy™3 dyes available
- Purified, duplexed, and ready to use

Application:
For monitoring siRNA delivery in cultured human, mouse, and rat cells by fluorescence microscopy or flow cytometry

Delivery Method:
siRNAs can be delivered into cultured cells by transfection or electroporation

The *Silencer*® Cy3 and FAM Dye-Labeled GAPDH and Negative Control #1 siRNAs provide an easy way to monitor siRNA uptake by fluorescence microscopy or flow cytometry. The sequences of these siRNAs are identical to Ambion's popular GAPDH (human) and Negative Control #1 sequences, which have been used extensively as controls by researchers worldwide. Each has been labeled at the 5' end of the sense strand with either the fluorescein derivative, FAM (5'-carboxyfluorescein; excitation maximum = 494 nm, emission maximum = 520 nm), or Cy3 (excitation maximum = 547, emission maximum = 563 nm) to make them highly fluorescent when excited by the appropriate wavelength of light.

Silencer Cy3 and FAM Labeled siRNA Controls are provided purified, annealed and ready to use. Each 5 nmol aliquot is supplied in a stable dried format along with a tube of nuclease-free water.

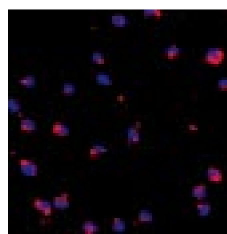

Figure 20. Monitoring siRNA Delivery with Cy™3 Dye-labeled Negative Control #1 siRNA. *Silencer*® Cy3 Labeled Negative Control siRNA (1 µg) was electroporated into HUVEC cells in siPORT™ Electroporation Buffer (Cat #AM8990). Nuclei were stained with DAPI and cells were imaged by fluorescence microscopy.

ORDERING INFORMATION		
Cat#	Description	Size
AM4620	*Silencer*® FAM™ dye-labeled Negative Control #1 siRNA	5 nmol
AM4621	*Silencer*® Cy™3 dye-labeled Negative Control #1 siRNA	5 nmol
AM4622	*Silencer*® FAM™ dye-labeled GAPDH siRNA (human)	5 nmol
AM4623	*Silencer*® Cy™3 dye-labeled GAPDH siRNA (human)	5 nmol

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Silencer Cy3 labeled siRNAs are manufactured under license from Carnegie Mellon University under U.S. Patent Numbers 6,048,982 and 6,225,050 and related patents.
Cy3 is a trademark of Amersham Biosciences Ltd.

Silencer® siRNA Labeling Kits

For the Fluorescent Labeling of siRNA

- Compatible with both chemically and enzymatically synthesized siRNAs
- Label up to twelve siRNAs, on one or both strands
- Simple procedure that is complete in ~2 hours
- Cy™3 and Fluorescein (FAM™) Dye-Labeling Kits available

Application:
For labeling siRNA with Cy3 dye or fluorescein; labeled siRNAs can be used to track siRNA delivery into cells

The *Silencer*® Cy3 and FAM siRNA Dye-labeling Kits are ideal for fluorescently labeling siRNAs. Labeled siRNA can be used to analyze siRNA subcellular localization and stability, and to identify transfected cells. In addition, fluorescently labeled siRNA is particularly suited for use in double labeling experiments with a labeled antibody to correlate siRNA uptake with down-regulation of the target protein (Figure 21).

Simple Procedure for siRNA Labeling

The labeling procedure is simple. Just combine your siRNA with the suspended Labeling Reagent, incubate for 1 hr at 37°C and ethanol precipitate to remove unreacted Labeling Reagent. Two *Silencer* siRNA Labeling Kits are available: Cy3 and FAM Dyes. Each kit comes with all the reagents necessary to label up to 65 µg of siRNA, a GAPDH siRNA control for labeling, and a detailed Instruction Manual. Labeling reactions are scalable, but typically twelve 5 µg aliquots of siRNA are labeled per kit. Note that the labeling procedure can be adapted to fluorescently label almost any RNA, including the long dsRNAs used in *Drosophila* and *C. elegans* gene silencing experiments.

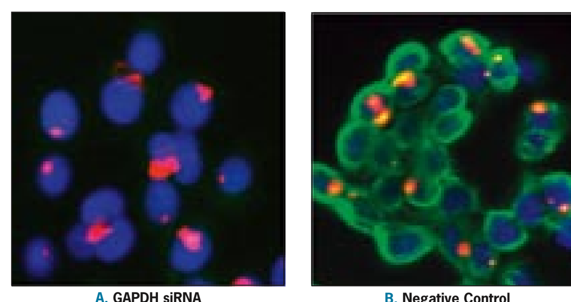

Figure 21. Correlating Gene Silencing with siRNA Uptake. An siRNA to human GAPDH and a nontargeting control siRNA were fluorescently labeled with Cy™3 Dye using the *Silencer*® siRNA Labeling Kit and transfected into HeLa S3 cells. Cells were analyzed by fluorescence microscopy with an anti-GAPDH antibody. Red: Cy3 dye-labeled siRNA; Blue: DAPI stained nuclei; Green: GAPDH protein. A. siRNA silencing of GAPDH expression. B. Nontargeting control siRNA has no effect on GAPDH protein levels.

ORDERING INFORMATION		
Cat#	Description	Size
AM1632	*Silencer*® siRNA Labeling Kit - Cy™3 Dye	65 µg labeled siRNA
AM1634	*Silencer*® siRNA Labeling Kit - FAM™ Dye	65 µg labeled siRNA

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The Cy3 and Fluorescein Labeling Reagents are manufactured for Ambion by Mirus Corporation.

Antibodies Matched to *Silencer*® Control siRNAs

Application:
For detecting and quantifying proteins by Western blotting, immunofluorescence, or other immunological technique; useful for monitoring siRNA induced knockdown at the protein level

To complement Ambion's line of products for siRNA research, high quality antibodies are available for a few key proteins. These antibodies are ideal for monitoring siRNA induced knockdown at the protein level by immunofluorescence and/or Western analysis. Some of the antibodies (including anti-GAPDH, anti-c-myc, anti-ß-actin, and anti-Ku) are ideal for use in double label experiments with fluorescently labeled siRNA to correlate the uptake of siRNA with the down regulation of target expression (Figures 21 and 22). Other antibodies such as anti-PCNA, anti-p53, and anti-Stat1, can be used to analyze target protein expression by Western analysis. For additional information and data, see the table below and the Ambion website at **www.ambion.com/catalog/CatNum.php?4300.**

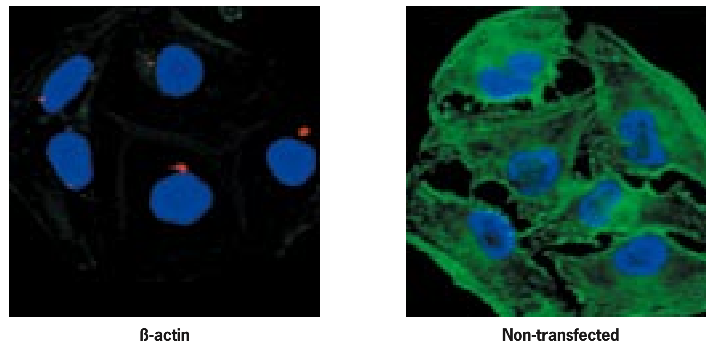

Figure 22. Following the Silencing of ß-actin. An siRNA targeting ß-actin was prepared by in vitro transcription with the *Silencer*® siRNA Construction Kit and then labeled with Cy™3 dye using the *Silencer* siRNA Labeling Kit. The labeled siRNA was transfected into HeLa cells and cells were analyzed 96 hours later. Green: ß-actin protein detected with anti-ß-actin (Ambion) and a fluorescein labeled secondary antibody. Red: Cy3 labeled siRNA. Blue: DAPI stained nuclei.

ORDERING INFORMATION

Cat#	Description	Size
AM4300	anti-GAPDH, mouse monoclonal 6C5	100 µg
AM4302	anti-β-actin, mouse monoclonal AC-15	100 µg
AM4304	anti-p53, mouse monoclonal DO-7 + BP53-12	25 µg
AM4309	anti-Cyclophilin A, rabbit polyclonal	10 µg

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Characteristics of Ambion's Primary Antibodies for siRNA Research.

Target	Target Species	Clone	Ig Subtype	Format	Uses
β-Actin	Human, Bovine, Sheep, Pig, Cat, Rabbit, Dog, Guinea Pig, Chicken, Carp, Leech, Fruit Fly	AC-15	IgG_1	Ascites Fluid	I
Cyclophilin A	Human, Mouse, Rat	N/A	N/A	N/A	W
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Human, Rabbit, Mouse, Fish, Frog, Chicken	6C5	IgG_1	Protein A purified	I, W
p53	Human	DO-7 + BP53-12	IgG_{2b} + IgG_{2a}	Protein A purified	W

Silencer® siRNA Starter Kit

Complete Kit for Inducing and Monitoring RNAi

- The perfect choice for those beginning siRNA experiments
- Includes transfection agent, siRNAs, and three different systems for assessing knockdown
- For use with human, mouse, and rat cells

Application:
For inducing and monitoring RNAi using GAPDH and negative control siRNAs

Delivery Method:
Transfection

Just starting siRNA experiments in human, mouse, or rat cells? The *Silencer*® siRNA Starter Kit is perfect for researchers new to RNAi. It provides all of the reagents, protocols, and instructions necessary to get underway with siRNA transfection and ensure successful gene knockdown experiments (Figure 23).

Transfection Agent

The *Silencer* siRNA Starter Kit includes a high quality, lipid-based transfection agent, siPORT™ *NeoFX*™ Transfection Agent, that effectively delivers siRNAs to a broad range of cell lines. This reagent is not sensitive to serum and efficiently transfects low concentrations of siRNA. See page 29 for further details.

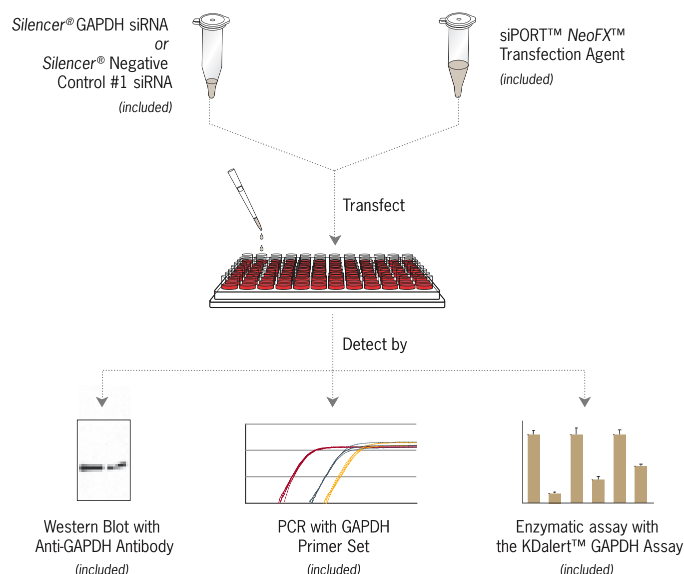

Figure 23. Complete Kit for Silencing and Detecting GAPDH. The *Silencer*® siRNA Starter Kit is ideal for your first RNAi experiment, as well as for optimizing and verifying siRNA delivery in human, mouse, and rat cells. Although a 96-well plate is shown, experiments can also be performed in 24-well or 6-well plates.

Control siRNAs

The kit also includes a well-characterized GAPDH siRNA, which has been shown to strongly down regulate GADPH in human, mouse, and rat cells, as well as a nontargeting negative control siRNA, *Silencer* Negative Control #1 siRNA. Because GAPDH is ubiquitously expressed and is easy to detect at both the mRNA and protein level, it is an ideal target for a positive control siRNA.

Three Detection Systems

The kit includes three different systems for monitoring GAPDH siRNA-induced knockdown. An anti-GAPDH antibody is provided to assess GAPDH protein levels by Western blot or immunofluorescence. Ambion's KDalert™ GAPDH Assay is also provided to quickly measure GAPDH enzymatic activity. This assay can also be used to assess cell viability. Finally, RT-PCR primers are included, for real-time quantitative RT-PCR detection of GAPDH mRNA.

The Perfect Complement: *Silencer* Pre-designed siRNAs

When you combine the *Silencer* Starter Kit with *Silencer* Pre-designed siRNAs—guaranteed-to-silence siRNAs for human, mouse, or rat genes (see page 16) — you have a complete solution for RNAi experiments in cultured cells.

ORDERING INFORMATION

Cat#	Description	Size
AM1640	*Silencer*® siRNA Starter Kit	1 kit

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Silencer® siRNA Construction Kit

For the Enzymatic Preparation and Purification of siRNA

- Make and purify up to 15 siRNAs in less than 24 hours
- Hundreds of transfections per reaction

Application:
For preparing siRNAs that will be used to induce transient RNAi in cultured mammalian cells

Delivery Method:
siRNAs can be delivered into cultured cells by transfection or electroporation

The *Silencer*® siRNA Construction Kit provides an optimized in vitro transcription method for the preparation of siRNAs, as well as reagents to purify the siRNAs after synthesis. Templates for the *Silencer* siRNA Construction Kit reactions are produced from two inexpensive DNA oligonucleotides encoding the desired siRNA strands. The oligonucleotides are each annealed to the included T7 promoter primer, and subjected to a fill-in reaction with Klenow fragment to generate a double-stranded transcription template. After transcription of each strand, the reactions are combined for annealing. The siRNA preparation is then treated with DNase to remove the DNA template and RNase to remove the 5' terminal triphosphates and polish the ends of the double-stranded RNA. The final step is column purification. The entire procedure requires little hands on time and can be completed in less than 24 hours.

ORDERING INFORMATION

Cat#	Description	Size
AM1620	*Silencer*® siRNA Construction Kit	15 siRNA synthesis rxns
AM4800	*Silencer*® GAPDH siRNA Control Template Kit	10 rxns (includes negative control template)
AM4850	Negative Control #1 siRNA Templates	10 rxns

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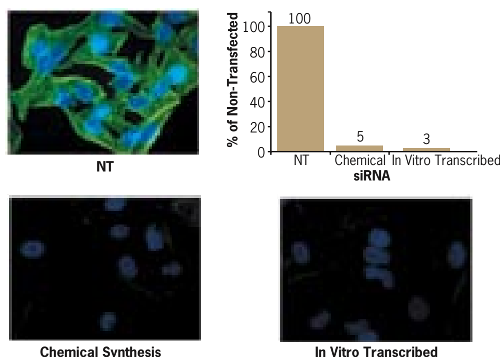

Figure 24. Gene Silencing with Chemically Synthesized and In Vitro Transcribed siRNAs. siRNAs targeting ß-actin were prepared by chemical synthesis or by in vitro transcription using the *Silencer*® siRNA Construction Kit. HeLa cells were transfected 24 hours after plating using 2 µL siPORT™ *Lipid* Agent and a final siRNA concentration of 75 nM. Immunofluorescence analysis was performed 96 hr post transfection using mouse anti-Human ß-actin primary antibody (Cat# AM4302) and a FITC conjugated anti-mouse IgG secondary antibody. Both siRNA preparation methods resulted in >95% reduction in ß-actin protein levels.

Silencer® siRNA Cocktail Kit (RNase III)

For the Enzymatic Preparation of siRNA Populations

- Eliminates the need for individual siRNA design and testing
- Creates a population of siRNAs
- Entire procedure complete in 1 day

Application:
For preparing siRNA mixtures that will be used to induce transient RNAi in mammalian cultured cells

Delivery Method:
siRNAs can be delivered into cultured cells by transfection or electroporation

The *Silencer*® siRNA Cocktail Kit provides reagents for the generation of siRNA populations, or cocktails, by enzymatic synthesis of long dsRNA followed by digestion by RNase III (Figure 25).

Preparing siRNA cocktails with the *Silencer* siRNA Cocktail Kit is fast and simple. First, long dsRNA complementary to the target of interest is generated by in vitro transcription from a DNA template with opposing T7 phage polymerase promoter primers. Alternatively, the dsRNA can be prepared from two separate templates with promoters on opposite ends of the region to be transcribed. Instructions for preparing such templates are provided in the kit Instruction Manual. After purification using the included columns, the dsRNA is digested by RNase III to create a population of siRNAs. Any residual long dsRNA is removed with the included siRNA Purification Units. The siRNA cocktail is then ready for transfection.

ORDERING INFORMATION

Cat#	Description	Size
AM1625	*Silencer*® siRNA Cocktail Kit (RNase III)	20 rxns
AM2290	RNase III	250 U

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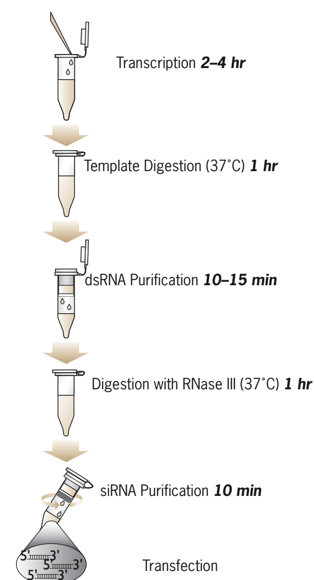

Figure 25. *Silencer*® siRNA Cocktail Kit Protocol.

siPORT™ *NeoFX*™ Transfection Agent

- Works with a broad range of cell lines
- Produces consistent results, lot-to-lot, plate-to-plate, and well-to-well
- Allows use of low siRNA concentrations, which minimize nonspecific effects
- Reverse transfects cells as they are plated, saving a day

Application:
For delivering siRNA into cultured mammalian cells; recommended for immortalized, adherent cell lines

siPORT™ *NeoFX*™ Transfection Agent is a versatile lipid-based reagent that efficiently and reproducibly delivers siRNA into many different mammalian adherent cell types (Figure 26). The streamlined protocol can be adapted to a wide range of cells and experimental designs, including high throughput applications.

Save a Day by Transfecting Cells as They Are Plated

The siPORT *NeoFX* Transfection Agent protocol is faster and easier than traditional transfection protocols. Just add siPORT *NeoFX* Transfection Agent to your diluted siRNA, incubate to form transfection complexes, add the complexes to the culture wells, and overlay with cells. This streamlined "reverse transfection" protocol saves a day of valuable time when compared to traditional plated transfection procedures. And because the transfection complexes are active and stable even in the presence of serum, it is not necessary to remove or replace media following transfection.

High Performance Delivery Allows Use of Low siRNA Concentrations

siRNA concentrations of 100 nM or higher can lead to nonspecific changes in gene expression (off-target effects) in mammalian cultured cells. Reducing the amount of siRNA used for transfections to 1–20 nM minimizes these nonspecific effects, while still providing effective silencing of the target gene. Ambion scientists specifically developed siPORT *NeoFX* Transfection Agent for efficient transfection of low concentrations of siRNA, and the reagent effectively delivers even picomolar amounts of siRNA (Figure 27).

Consistent, Reliable Results

A good transfection agent is expected to reproducibly deliver siRNAs to replicate samples, even across different lots of the reagent. But some transfection agents do not live up to this promise. siPORT *NeoFX* Transfection Agent is remarkably stable at various temperatures, which translates to reproducible siRNA delivery from day to day and experiment to experiment (Figure 28). Such characteristics are especially important in applications such as RNAi screening that require reliable high throughput siRNA delivery.

ORDERING INFORMATION		
Cat#	Description	Size
AM4510	siPORT™ *NeoFX*™ Transfection Agent	0.4 mL
AM4511	siPORT™ *NeoFX*™ Transfection Agent	1 mL

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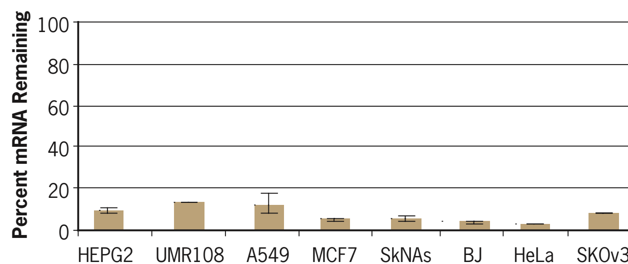

Figure 26. siPORT™ *NeoFX*™ Transfection Agent Effectively Delivers siRNA into Multiple Cell Types. *Silencer*® GAPDH siRNA or Negative Control #1 siRNA (10 nM) was transfected into the indicated cell types with siPORT *NeoFX* Transfection Agent. Shown are the remaining mRNA levels, as measured by real-time PCR 48 hr after transfection, as compared to negative control siRNA transfected cells.

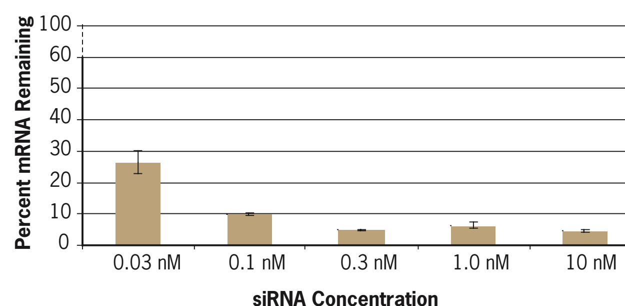

Figure 27. Efficient Transfection with Low siRNA Concentrations. HeLa cells were trypsinized and resuspended in growth media at a concentration of 4x10^{4} cells/mL. Transfection complexes were prepared using chemically synthesized GAPDH siRNA (0.03 nM to 10 nM, see graph above) or negative control siRNA (data not shown) and 2 µL siPORT™ *NeoFX*™ Transfection Agent. 48 hours after transfection, cells were harvested and analyzed by real-time RT-PCR for both GAPDH mRNA and 18S rRNA levels.

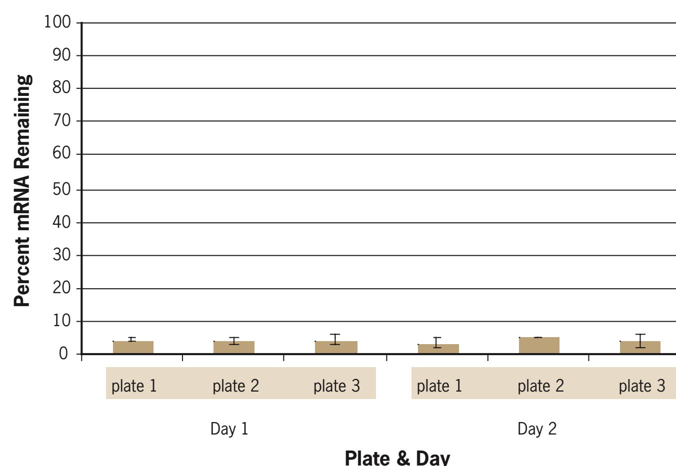

Figure 28. Consistent Day-to-day, Plate-to-Plate, and Well-to-Well Performance of siPORT™ *NeoFX* Transfection Agent. HeLa cells (8000 cells/well) were transfected in 96 well plates using siPORT *NeoFX* Transfection Agent and either *Silencer*® GAPDH siRNA (10 nM) or a negative control siRNA. Remaining GAPDH expression was quantified by real-time PCR. Each bar represents the mean of eight replicate wells. siPORT *NeoFX* demonstrates consistent transfection performance across multiple wells, among multiple plates, and on multiple days.

siPORT™ *Amine* Transfection Agent

- Optimized for efficient siRNA delivery into mammalian cultured cells
- Low cytotoxicity
- Easy to use
- Serum compatible

Application:
For delivering siRNA into cultured mammalian cells; recommended for immortalized, adherent cell lines

siPORT™ *Amine*, consisting of a mixture of polyamines, efficiently delivers siRNAs into a wide variety of mammalian cultured cells (Figures 29, 30) while demonstrating very low cytotoxicity. Because of its different formulation, siPORT *Amine* works better than siPORT *NeoFX* or siPORT *Lipid* for some cell types. siPORT *Amine* can be used in the presence of serum and is compatible with reverse transfection protocols, making it particularly easy to use.

ORDERING INFORMATION		
Cat#	Description	Size
AM4502	siPORT™ *Amine* Transfection Agent	0.4 mL
AM4503	siPORT™ *Amine* Transfection Agent	1 mL

siPORT™ Amine is manufactured for Ambion by Mirus Corporation.
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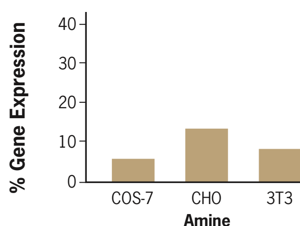

Figure 29. Delivery of siRNA by siPORT™ *Amine* Agent Induces RNAi. The indicated cell types were transfected with *Silencer*® GAPDH siRNA or negative control siRNA using siPORT *Amine* Transfection Agent. Gene silencing effects were monitored by Northern blot using the NorthernMax®-Gly Kit (Cat #AM1946). Bars indicate gene expression levels 48 hr after delivery of the gene-specific siRNA relative to negative control samples.

siPORT™ *Lipid* Transfection Agent

Application:
For delivering siRNA into cultured human cells; primarily for immortalized, adherent cell lines

siPORT™ *Lipid* Agent comprises a mixture of cationic and neutral lipids and successfully delivers siRNAs into many different mammalian cultured cells. This reagent works best in the absence of serum and is well suited to protocols in which the cells are plated a day prior to transfection.

Please note that the cell line compatibility of siPORT *Lipid* is similar to that of siPORT *NeoFX* Transfection Agent (page 31). For most cell types, Ambion recommends siPORT *NeoFX* Transfection Agent over siPORT *Lipid*, as siPORT *NeoFX* Transfection Agent consists of an improved, easy to use lipid formulation compatible with standard and reverse transfection protocols.

ORDERING INFORMATION		
Cat#	Description	Size
AM4504	siPORT™ *Lipid* Transfection Agent	0.4 mL
AM4505	siPORT™ *Lipid* Transfection Agent	1 mL

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Silencer® siRNA Transfection II Kit

- Ideal for those new to RNAi and for optimizing siRNA delivery
- Includes siPORT™ *Amine* and siPORT™ *NeoFX*™ Transfection Agent for broad cell line compatibility
- Ready-to-transfect *Silencer*® GAPDH and Negative Control siRNAs included for easy protocol optimization
- Combine with the new KDalert™ GAPDH Assay for a complete siRNA delivery optimization solution

Application:
For determining conditions for efficient delivery of siRNA into cultured human, mouse, and rat cells

The first step when optimizing siRNA transfection conditions for a new cell type is to test siRNA delivery efficiency using different transfection agents. The *Silencer* siRNA Transfection II Kit is the ideal choice for optimizing siRNA transfections in any size plate. The kit includes siPORT *NeoFX* and siPORT *Amine* Agents—Ambion's two popular siRNA transfection agents—as well as *Silencer* GAPDH siRNA and *Silencer* Negative Control #1 siRNA, two highly validated control sRNAs designed for use in human, mouse, and rat cells. The kit makes it easy to identify the appropriate siRNA delivery reagent and conditions for your cell type. It is also the recommended kit for those just starting siRNA experiments.

Delivery efficiency of the *Silencer* GAPDH siRNA can be assessed by monitoring GAPDH knockdown at the mRNA level by RT-PCR or Northern analysis. Alternatively, GAPDH knockdown can be monitored at the protein level using the new KDalert GAPDH Assay Kit (page 24) or with a monoclonal antibody to GAPDH (page 26).

ORDERING INFORMATION		
Cat#	Description	Size
AM1631	*Silencer*® siRNA Transfection II Kit	2 x .04 mL + controls
AM1639	KDalert™ GAPDH Assay Kit	375 rxns
AM4300	anti-GAPDH, mouse monoclonal 6C5	100 µg

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Cell Line	Mammalian Cell Type	Recommended Reagent
293	Human Kidney Epithelial, Transformed	siPORT™ *Amine* Agent
A549	Human Lung Carcinoma	siPORT™ *NeoFX*™ Agent
BJ	Human Foreskin Fibroblast	siPORT™ *NeoFX*™ or siPORT™ *Amine* Agent
CHO-K1	Hamster Ovary Epithelial	siPORT™ *Amine* Agent
COS-7	Monkey Kidney Fibroblast, Transformed	siPORT™ *Amine* Agent
HEP-G2	Human Hepatocellular Carcinoma	siPORT™ *NeoFX*™ Agent
HeLa	Human Cervix Adenocarcinoma	siPORT™ *NeoFX*™ Agent
HeLa-S3	Human Cervix Adenocarcinoma	siPORT™ *NeoFX*™ Agent
HT-29	Human Colorectal Adenocarcinoma	siPORT™ *NeoFX*™ or siPORT™ *Amine* Agent
MCF-7	Human Breast Adenocarcinoma	siPORT™ *NeoFX*™ Agent
NIH/3T3	Mouse Fibroblast	siPORT™ *Amine* Agent
SKNAS	Bone Marrow Neuroblastoma	siPORT™ *NeoFX*™ Agent
SKOv3	Ovarian Carcinoma	siPORT™ *NeoFX*™ Agent
UMR106	Rat Osteosarcoma	siPORT™ *NeoFX*™ Agent

Figure 30. Recommended siPORT™ Transfection Agent for High Efficiency Transfection.
See pages 46–47 for detailed transfection conditions.

siPORT™ siRNA Electroporation Kit and Buffer

For Delivery of siRNAs into Primary Cells

- Gentle on cells—high viability after electroporation
- Efficient siRNA delivery provides superior knockdown efficiency
- Successful with both adherent and suspension cells
- Compatible with most electroporators

Application:
For delivering siRNA into mammalian primary cells and cells in suspension by electroporation

An Electroporation Buffer that Protects Cells

Electroporation has become the method of choice for delivering siRNA into primary cells and cells in suspension. One key to successful electroporation, however, is the presence of a buffer designed to protect cells during application of the electrical pulse. Use of a non-optimized buffer is one of the primary reasons for excessive cell death seen with many electroporation protocols.

siPORT™ siRNA Electroporation Buffer is specially formulated to enhance cell viability during and after electoporation of siRNAs. Validated for use with most commonly used electroporators such as the BioRad® Gene Pulser Xcell™ and BTX ECM 830, as well as with Ambion's siPORTer™-96 Electroporation Chamber, the buffer minimizes sample heating, which leads to less stress on the cells during electroporation. In addition, the buffer's components emulate the natural cytoplasmic composition of cells, which accelerate pore resealing after electroporation. The result is efficient siRNA delivery and high cell viability with even difficult-to-transfect cells, such as primary cells and cells in suspension.

Ambion provides siPORT siRNA Electroporation Buffer as a stand-alone product. The stand-alone siPORT siRNA Electroporation Buffer contains enough reagents to perform 240 electroporations using standard 1 mm electroporation cuvettes.

A Complete System for Electroporating siRNAs into Cells

The siPORT siRNA Electroporation Kit makes it easy to optimize siRNA electroporation conditions by providing the tools necessary to test and optimize siRNA electroporation parameters. Each kit contains siPORT siRNA Electroporation Buffer, Cy™3 dye-labeled *Silencer*® Control siRNA, *Silencer* GAPDH Control siRNA, and *Silencer* Negative Control siRNA. The amount of siPORT siRNA Electroporation Buffer contained in the kit is sufficient for performing 60 electroporations using standard 1 mm electroporation cuvettes. The Cy3 dye-labeled Control siRNA can be used to monitor and optimize siRNA uptake into target cells; the GAPDH Control siRNA can be used to optimize electroporation conditions in human, mouse, and rat cells; and the Negative Control siRNA can be used to confirm the specificity of the gene-specific siRNA and absence of nonspecific toxic effects.

Using the siPORT siRNA Electroporation Buffer, Ambion has optimized the electroporation conditions for several primary and difficult-to-transfect cells (Figure 31). For experimental details, as well as for an up-to-date list of cell lines and optimized electroporation conditions, see page 47 and visit the siRNA Delivery Resource at **www.ambion.com/siRNA/delivery**.

ORDERING INFORMATION

Cat#	Description	Size
AM1629	siPORT™ siRNA Electroporation Kit	60 rxns
AM8990	siPORT™ siRNA Electroporation Buffer	12 x 1.5 mL
AM8990G	siPORT™ siRNA Electroporation Buffer, Trial Size	1.5 mL
AM8991	siPORT™ siRNA Electroporation Buffer	20 mL

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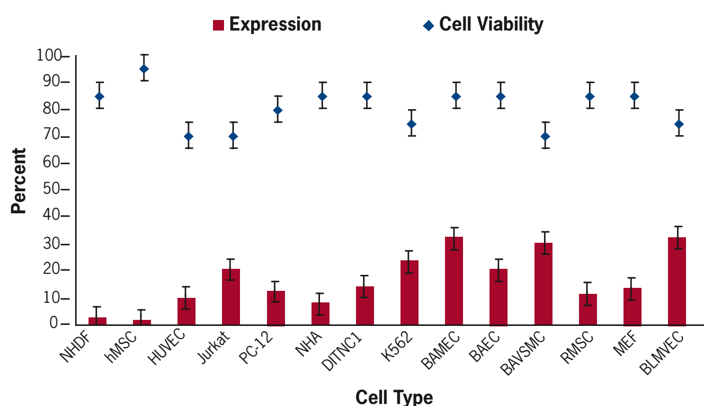

Figure 31. Reduction of GAPDH Gene Expression and Viability in Several Cell Types. Successful gene silencing and high cell viability was achieved in 11 primary cell types: human mesenchymal stem cells (hMSC), normal human keratinocytes (NHA), normal human dermal fibroblasts-neonatal (NHDF-Neo), rat astrocytes (DI TNC1), normal human umbilical vein endothelial cells (HUVEC), bovine aortic endothelial cells (BAEC), bovine aortic vascular smooth muscle cells (BAVSMC), bovine adrenal microvascular endothelial cells (BAMEC), mouse embryo fibroblast (MEF), rhesus monkey stem cells (RMSC), bovine lung microvascular endothelial cells (BLMVEC), and 3 hard-to-transfect cell types: Jurkat (human acute T-cells), K562 (human erythroleukemia cells) and PC12 (rat pheochromocytoma) cells. *Silencer*® GAPDH siRNA or Negative Control#1 siRNA (1.5 µg) was electroporated in triplicate using the Bio-Rad® Gene Pulser Xcell™ Pulse Generator and conditions optimized for each cell type. 48 hr post-electroporation, gene expression levels were monitored by real-time RT-PCR. Remaining gene expression was calculated relative to samples transfected with the Negative Control siRNA.

siPORTer™-96 Electroporation Chamber

- Efficient siRNA delivery into primary and other difficult-to-transfect cells
- Electroporation of up to 96 samples simultaneously
- High cell viability when used with siPORT™ siRNA Electroporation Buffer
- Highly reproducible results from well to well
- Easily cleaned electrodes— no need for consumable electroplates or cuvettes
- Designed for use with the Bio-Rad® Gene Pulser Xcell™ Pulse Generator

Application:
For simultaneous electroporation of siRNA into as many as 96 different samples—use with the BioRad Gene Pulser Xcell to deliver siRNA into mammalian primary cells and cells in suspension

Ambion's siPORTer™-96 Electroporation Chamber (Figure 32) enables high throughput electroporation of siRNAs into mammalian cells. Electroporation is becoming the method of choice for delivering siRNA into primary, suspension, and difficult-to-transfect cells, but single cuvette chambers seriously limit throughput. The siPORTer-96 Electroporation Chamber is designed for use with the popular Bio-Rad Gene Pulser Xcell Pulse Generator and Ambion's gentle-on-cells siPORT siRNA Electroporation Buffer. This electroporation chamber can deliver siRNA to up to 96 samples at a time.

Revolutionary Design Enables High Throughput Electroporation without Consumable Electroplates

Unlike other electroporation chambers, the siPORTer-96 Chamber does not require special electroplates or cuvettes, making use of the chamber surprisingly economical. Samples are pipetted directly into the wells of the lower electrode, and the upper electrode is placed on top. With the upper electrode in place, the upper and lower electrodes are in contact via a precisely spaced column of sample fluid. This allows the current to pass through samples when the power is applied, effecting electroporation. After removing the samples, the electrodes are simply cleaned with ethanol and then dried prior to loading the next set of samples.

Highly Reproducible Results from Well to Well

The siPORTer-96 Electroporation Chamber yields results that are highly reproducible from well to well. In Figure 33, 96 samples were electroporated using identical electroporation conditions. Half of the samples received an siRNA targeting GAPDH, and the other samples received a non-targeting negative control siRNA. The results were remarkably uniform.

Ambion scientists have used the siPORTer-96 Electroporation Chamber with many different cell lines (Figure 34). For the conditions employed, see Ambion's siRNA Delivery resource at **www.ambion.com/siRNA/delivery**.

What's Included

The siPORTer-96 Electroporation Chamber consists of a Main Housing Unit, a pre-assembled cartridge made up of 96 Well Electrode Plates and the Electrode Base Assembly, an extra set of 96 Well Electrode Plates, and siPORT siRNA Electroporation Buffer (see page 31). The siPORTer-96 Electroporation Chamber comes with a twelve month limited warranty.

ORDERING INFORMATION		
Cat#	Description	Size
AM13500	siPORTer™–96 Electroporation Chamber	1 device

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Figure 32. siPORTer™-96 Electroporation Chamber.

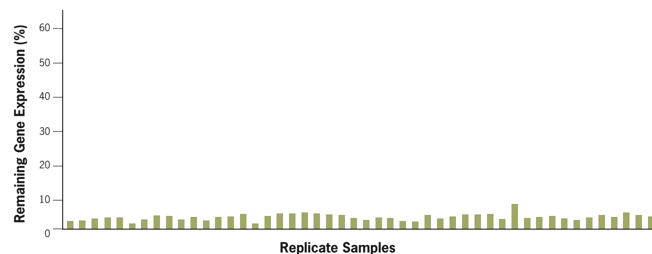

Figure 33. Reproducibility of siRNA Induced Knockdown After Electroporation Using the siPORTer™-96 Electroporation Chamber. *Silencer*® siRNAs in siPORT™ siRNA Electroporation Buffer were electroporated into Normal Human Dermal Fibroblasts-Neonatal cells (an adherent primary cell type) using the siPORTer-96 Electroporation Chamber powered by a BioRad® Gene Pulser Xcell™ Pulse Generator. Samples 1–48 were electroporated with *Silencer* GAPDH siRNA (1.0 µg) while samples 49–96 were electroporated with *Silencer* Negative Control #1 (1.0 µg). 48 hours later, target mRNA levels were analyzed by real-time RT-PCR. Remaining GAPDH mRNA levels for each GAPDH siRNA-treated well are expressed relative to the average GAPDH mRNA levels in the negative control siRNA-treated wells.

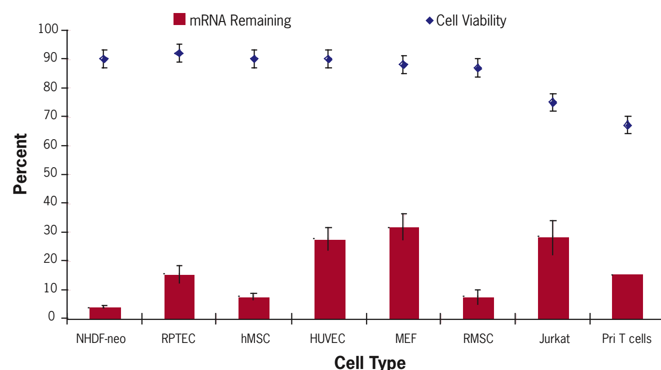

Figure 34. Electroporation with the siPORTer™-96 and siPORT™ siRNA Electroporation Buffer Results in Efficient siRNA Delivery and High Cell Viability. siRNA targeting GAPDH or a non-targeting siRNA (1.5 µg) in siPORT siRNA Electroporation Buffer was electroporated into 8 different cell types that are typically very difficult to transfect using lipid-based methods. 48 hr later, the cells were harvested and analyzed by real time RT-PCR. Remaining GAPDH mRNA levels are expressed relative to GAPDH mRNA levels in negative control siRNA treated samples. Cell viability is expressed relative to nontreated samples.

p*Silencer*™ siRNA Expression Vectors

Plasmid Vectors for Inducing RNAi

- Provides efficient silencing of target genes by RNA interference
- Expresses siRNA within mammalian cells using a U6 or H1 Polymerase III promoter or a CMV Polymerase II promoter
- Permits selection and long-term RNAi experiments through antibiotic resistance genes

Application:
For transient and long-term gene silencing in cultured cells

Delivery Method:
siRNA expression vectors can be delivered into cultured cells by transfection or electroporation

The p*Silencer*™ siRNA Expression Vectors are a family of plasmids designed for inducing RNAi by the expression of siRNA within mammalian cultured cells. Each p*Silencer* Vector has an RNA polymerase (pol) promoter driving expression of the siRNA precursor, an ampicillin resistance gene, and an *E. coli* origin of replication. To elicit silencing, a small DNA insert encoding a short hairpin RNA (shRNA) targeting the gene of interest is cloned into the vector downstream of the promoter. Once transfected into mammalian cells, the insert-containing vector expresses the shRNA, which is rapidly processed by the cellular machinery into siRNA.

Choice of Promoters

Ambion provides siRNA expression vectors with several different promoters. In general, all are suitable for human, mouse, and rat cells. However, silencing results may vary depending on the cell type used and the particular siRNA sequence being expressed. For your first siRNA expression vector, we recommend the p*Silencer* 2.1-U6 vector.

p*Silencer* 1.0-U6 Vector

The p*Silencer* 1.0-U6 Vector includes the mouse U6 Pol III promoter. This vector was developed by Shi and colleagues at Harvard Medical School [Sui et al., (2002) *PNAS* **99:** 5515–5520].

p*Silencer* 2.0-U6 / 2.1-U6 / 3.0-H1 / 3.1-H1 Vectors

p*Silencer* 2.0-U6 and p*Silencer* 3.0-H1 Vectors feature two different RNA Pol III promoters. p*Silencer* 2.0-U6 Vector contains the human U6 promoter that has been used extensively to express siRNAs and ribozymes in mammalian cells. p*Silencer* 3.0-H1 Vector features the H1 RNA promoter (H1 RNA is a component of RNase P). p*Silencer* 2.1-U6 Vector and p*Silencer* 3.1-H1 Vectors feature the same promoters, but also include antibiotic resistance genes for selection with G418 (neomycin analog), puromycin, or hygromycin.

p*Silencer* 4.1-CMV Vectors

p*Silencer* 4.1-CMV vectors carry a modified RNA polymerase II human cytomegalovirus (CMV) immediate-early promoter and an optimized SV40 polyadenylation signal. This pol II promoter tolerates strings of 4 or more U's within the siRNA sequence, unlike pol III which will terminate transcription after incorporation of a stretch of U's. In addition, the CMV promoter does not interfere with other transcription events, making it easier to perform long-term gene silencing studies. These vectors also include neomycin, puromycin, or hygromycin resistance genes for antibiotic selection.

Linearized Vectors, Ready for Cloning

All of the p*Silencer* siRNA Expression Vectors (except p*Silencer* 1.0 Vector) include four components: (1) linearized and purified vector ready for ligation; (2) an siRNA encoding insert that can be used as a positive control; (3) a circular, negative control p*Silencer* Vector that expresses an siRNA with limited homology to any known sequences in the human, mouse, and rat genomes; and (4) 1X DNA Annealing solution to prepare DNA oligonucleotides for ligation.

ORDERING INFORMATION

p*Silencer* Vector	Promoter	Linearized with	Puromycin	Hygromycin	Neomycin (G418)	Positive Control	Negative Control*	Cat#	Size
1.0-U6 (circular)	Mouse U6	—				—	—	AM7207	20 µg
2.0-U6	Human U6	BamH I/ Hind III				GAPDH insert	✓	AM7209	20 rxns
2.1-U6 puro	Human U6	BamH I/ Hind III	X			GFP insert	✓	AM5762	20 rxns
2.1-U6 hygro	Human U6	BamH I/ Hind III		X		GFP insert	✓	AM5760	20 rxns
2.1-U6 neo	Human U6	BamH I/ Hind III			X	GFP insert	✓	AM5764	20 rxns
3.0-H1	Human H1	BamH I/ Hind III				GAPDH insert	✓	AM7210	20 rxns
3.1-H1 puro	Human H1	BamH I/ Hind III	X			GFP insert	✓	AM5768	20 rxns
3.1-H1 hygro	Human H1	BamH I/ Hind III		X		GFP insert	✓	AM5766	20 rxns
3.1-H1 neo	Human H1	BamH I/ Hind III			X	GFP insert	✓	AM5770	20 rxns
4.1-CMV puro	CMV	BamH I/ Hind III	X			GAPDH insert	✓	AM5775	20 rxns
4.1-CMV hygro	CMV	BamH I/ Hind III		X		GAPDH insert	✓	AM5777	20 rxns
4.1-CMV neo	CMV	BamH I/ Hind III			X	GAPDH insert	✓	AM5779	20 rxns

Negative controls are comprised of the corresponding circular vector encoding a non-targeting siRNA.

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These products are the subject of the following patents: U.S. Patent No. 6,573,099 and foreign counterparts co-owned by Benitec Australia Ltd.

These products are covered by United States Patent Application No. 10/195,034, and PCT patent application No. US02/22010, owned by the University of Massachusetts.

The pSilencer® 1.0-U6 Vector is the subject of a US Patent Application and is sold under licensing arrangement with Harvard Medical School.

siPORT™ *XP-1* Transfection Agent

For Plasmid DNA and PCR Product Transfection

- Efficient delivery of plasmid DNA and PCR products
- Low toxicity
- Works in the presence or absence of serum
- Simple to use

Application:
For delivering plasmids and PCR products into cultured mammalian cells

siPORT *XP-1* agent is an easy-to-use reagent that efficiently delivers both plasmid DNA and PCR products into a variety of mammalian cell types. Comprised of a proprietary formulation of polyamines, the reagent exhibits low toxicity and can be used both in the presence or absence of serum. siPORT *XP-1* agent is particularly straightforward to use. Reagent/DNA complexes can be added directly to growing cells in serum-containing culture media, and the media can be left unchanged until the time of assay. In Figure 35, a plasmid engineered to express an siRNA to GAPDH, was transfected using siPORT *XP-1* agent into three different cell lines. In all three cell lines, GAPDH mRNA levels were reduced >80%, demonstrating the effectiveness of siPORT *XP-1* agent in this application.

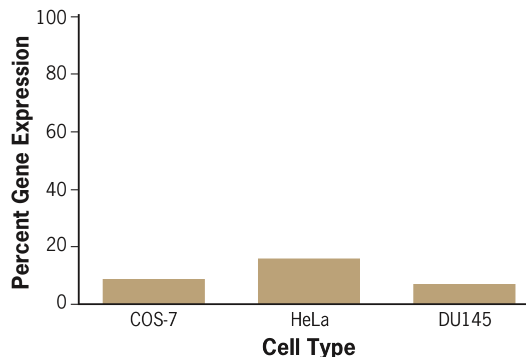

Figure 35. Gene Silencing with an siRNA Expression Plasmid Transfected with siPORT™ *XP-1* Agent. p*Silencer*™ 2.0-U6 Vector system was engineered to express an siRNA to GAPDH and then transfected into COS-7, HeLa, and DU145 cells with siPORT *XP-1* agent. 72 hr after transfection, RNA was isolated with the RNAqueous® Kit and GAPDH mRNA levels were analyzed by Northern blot using Ambion's NorthernMax®-Gly Kit. GAPDH mRNA expression levels were normalized to levels obtained from mock transfected cells.

ORDERING INFORMATION

Cat#	Description	Size
AM4506	siPORT™ *XP-1* Transfection Agent	0.4 mL
AM4507	siPORT™ *XP-1* Transfection Agent	1 mL

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p*Silencer*™ 5.1 Retro System

- Integrates into the host's genome—ideal for long term gene silencing
- Compatible with a broad range of mammalian cells and organisms
- Choice of human U6 and H1 polymerase III promoters
- Efficiently silences mammalian genes by RNA interference

Application:
For transient and long-term silencing in cultured cells.

Delivery Method:
Delivery of plasmid form into cultured cells by transfection or electroporation; delivery of viral form by infection.

An Effective Method for Long-Term Gene Silencing

Ambion's p*Silencer*™ 5.1 Retro System enables researchers to study the long-term effects of reduced gene expression in cell culture models, including difficult-to-transfect cell lines. Gene expression is reduced through the stable expression of an siRNA using an H1 or U6 promoter.

Suppression of Gene Expression in Primary Cells

A challenge for many researchers studying gene function is delivering siRNAs or siRNA expression vectors into primary cells—a problem alleviated with retroviral vectors. Ambion scientists delivered recombinant retroviruses bearing an siRNA insert targeting cyclophilin A into NHDF-neo cells, a primary cell line that is difficult to transfect, and achieved greater than 90% suppression of cyclophilin A gene expression (see **www.ambion.com/techlib/tn/121/22.html**).

Choice of U6 and H1 Polymerase III Promoters Allows for Optimal Gene Silencing

The p*Silencer* 5.1-Retro vectors are available with either the U6 or H1 RNA polymerase III promoters, which generate large amounts of transcript using relatively simple promoter and terminator sequences. This choice of promoter enables researchers to find the optimal promoter for their gene of interest, thereby achieving the greatest possible reduction in gene expression.

Ready-to-use Vectors Supplied with Positive and Negative Controls

The p*Silencer* 5.1-Retro vector system is supplied with (1) *Bam* H1 and *Hind* III-linearized and purified vector ready for ligation; (2) a DNA insert encoding a cyclophilin A gene-specific siRNA; (3) a circular, negative control p*Silencer* vector that expresses a nontargeting control siRNA; and (4) 1X DNA Annealing Solution. The packaging cell line is not included.

ORDERING INFORMATION		
Cat#	Description	Size
AM5782	p*Silencer*™ 5.1-U6 Retro Vector System	20 rxns
AM5784	p*Silencer*™ 5.1-H1 Retro Vector System	20 rxns

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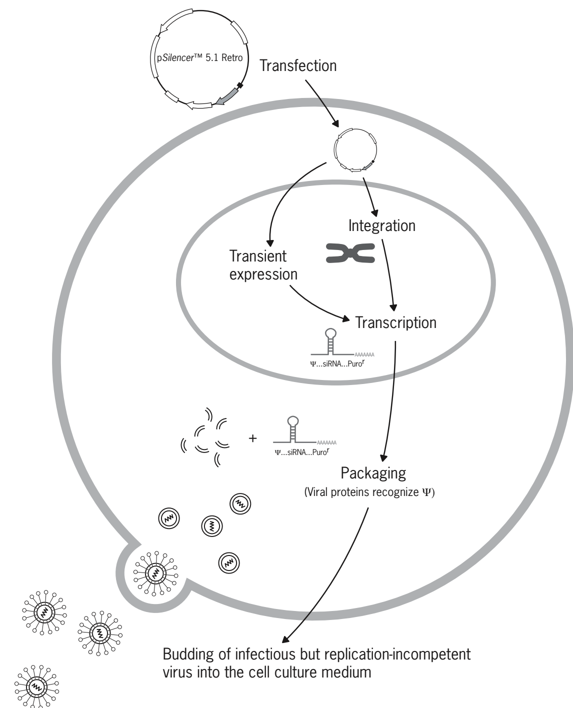
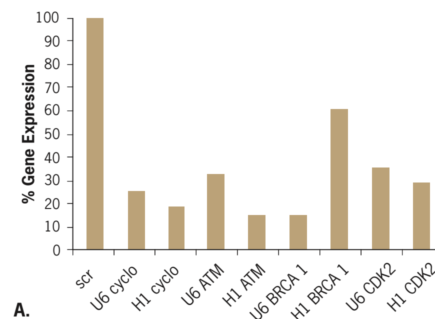


Figure 36. Production of Recombinant Viral Particles in a Retroviral Packaging Cell Line



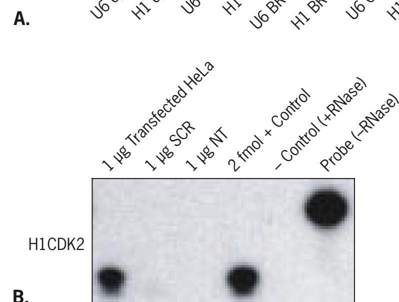


Figure 37. Knockdown of Endogenous Genes in HeLa Cells Using Retroviral-mediated Delivery of siRNA and Analysis of Expression of H1 Regulated CDK2 siRNA. Retroviral vectors containing either the H1 or U6 promoter and siRNA inserts were transfected into a retrovirus packaging cell line in 6 well plates. Three days following transfection, the virus was collected and used to infect HeLa cells. **(A)** The HeLa cells were placed under puromycin selection (4 days). After two months of continuous growth, RNA was purified from the cells and analyzed by real-time PCR for target gene expression levels. Data were normalized to expression by negative control siRNA transfected cells. Standard deviations were all below 5%. **(B)** The expression levels the CDK2 siRNAs regulated by the H1 promoter (H1 CDK2) was monitored using the *mir*Vana™ miRNA Isolation and Detection Kits (Cat# AM1560 and AM1552).

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These products are covered by United States Patent Application No. 10/195,034, and PCT patent application No. US02/22010, owned by the University of Massachusetts.

p*Silencer*™ adeno 1.0-CMV System

- Efficient delivery into a broad range of mammalian cells and organisms
- Ideal for difficult-to-transfect cells, including many primary and terminally differentiated cells
- Proven system demonstrated to specifically silence target genes in both cultured cells and in animals [1]
- Does not integrate into host genome so little chance of insertional mutagenesis

Application:
For transient silencing by RNAi in cultured cells

Delivery Method:
Delivery of plasmid form into cultured cells by transfection or electroporation; delivery of virus by infection

Ambion's p*Silencer*™ adeno 1.0-CMV System combines the advantages of adenoviral vectors with an efficient siRNA expression system. With p*Silencer* adeno, you can easily deliver an siRNA expressing construct into a variety of mammalian cells and organisms. The modified CMV promoter in the vector efficiently expresses hairpin siRNAs, which are effective at inducing RNAi. The p*Silencer* adeno 1.0-CMV Vector was developed by Beverly Davidson and colleagues and has been used to silence GFP and endogenous ß-glucuronidase in HeLa cells and adult mice [1]. Figure 39 shows the silencing of GAPDH in HeLa cells after infection with p*Silencer* adeno 1.0-CMV engineered to express the corresponding siRNA.

Efficient Delivery via Viral Infection

Adenoviruses are popular gene delivery vehicles because they efficiently transduce many different cell types, including terminally differentiated cells. Infection is independent of cell cycle, so adenoviruses can be used to express RNA in both dividing and non-dividing cells. Integration of the adenoviral DNA into the host genome is rare, which means that there is little chance of insertional mutagenesis. Because of this feature and the fact that most recombinant adenoviruses elicit an immune response in animal systems, these viral vectors are appropriate only for transient RNA expression.

The Complete Adenovirus Production System

The p*Silencer* adeno 1.0-CMV System includes everything needed to produce five preparations of recombinant adenovirus, except the specific siRNA encoding oligonucleotides to be cloned into the vector and the HEK 293 packaging cells (HEK 293 cells are available from several sources, including ATCC). The kit includes linearized Shuttle Vector 1.0-CMV (20 rxns), negative control shuttle vector that encodes a non-targeting siRNA sequence, a positive control oligonucleotide insert that encodes a GAPDH siRNA, and an adenoviral backbone that includes a *LacZ* sequence for ready monitoring of transfection efficiency. Also included are reagents for transfecting the HEK 293 cells with the shuttle and backbone vectors, forward and reverse sequencing primers to verify clones, and 5X Annealing Buffer for preparing the siRNA encoding oligonucleotides for ligation. An overview of the procedure for making adenoviral shRNA vectors is shown in Figure 38.

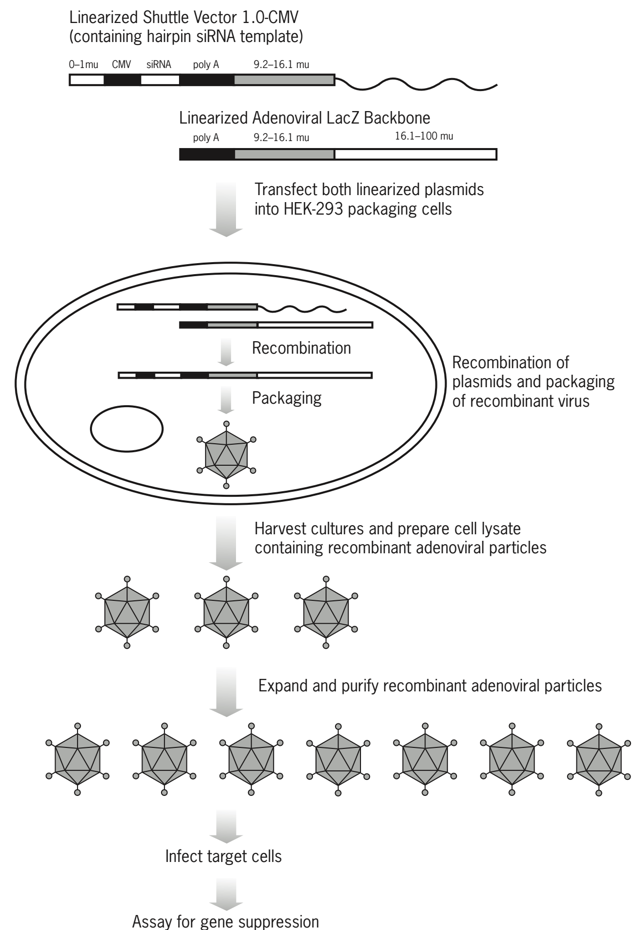

Figure 38. p*Silencer*® adeno System Protocol.

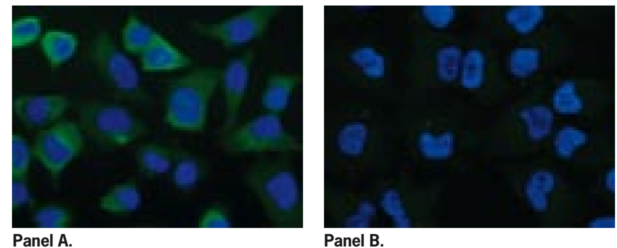

Figure 39. Silencing of GAPDH Induced by p*Silencer*™ adeno System. HeLa cells were infected at an MOI of 80 with adenovirus derived from the p*Silencer* adeno 1.0-CMV System. The virus was designed to express a GAPDH siRNA **(Panel B)** or a nontargeting negative control siRNA **(Panel A)**. The media was changed 4 hours after infection and cells were analyzed after 72 hours. Immunofluorescence was performed using a mouse anti-GAPDH antibody. Green: GAPDH protein. Blue: DAPI stained nuclei.

ORDERING INFORMATION

Cat#	Description	Size
AM5790	p*Silencer*™ adeno 1.0-CMV System	5 virus preparations

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These products are the subject of the following patents: U.S. Patent No. 6,573,099 and foreign counterparts co-owned by Benitec Australia Ltd.

These products are covered by United States Patent Application No. 10/195,034, and PCT patent application No. US02/22010, owned by the University of Massachusetts.

References

1. Xia H, Mao Q, Paulson HL, and Davidson BL (2002) siRNA-mediated gene silencing in vitro and in vivo. *Nat Biotech* **20**: 1006-1010.

MEGAscript® RNAi Kit

High Yield Transcription Kit for dsRNA Preparation and Purification

- Produces dsRNA ideal for RNAi experiments in non-vertebrate systems
- Synthesizes 50–80 µg or more dsRNA per reaction
- Contains everything needed for efficient transcript clean up
- Synthesizes over 1 mg of dsRNA

Application:
For generating long dsRNA designed to initiate RNAi in *C. elegans*, *Drosophila*, and other nonmammalian systems

Delivery Method:
Delivery of dsRNA into nonmammalian organisms by injection, soaking, or transfection, depending on organism and cell type.

The ability to generate large amounts of purified, double-stranded RNA (dsRNA) is essential for the success of RNAi experiments in non-mammalian systems. The MEGAscript® RNAi Kit, based on Ambion's patented MEGAscript large scale transcription technology, synthesizes 50 µg or more of dsRNA up to 1.2 kb in length in a single transcription reaction (see Figure 40). Each DNA template is transcribed hundreds of times by T7 RNA polymerase, so that more than a milligram of RNA can be produced from a single kit.

DNA of either PCR or plasmid origin can be used as a template for the transcription reaction. The sense and antisense strands can be synthesized in separate reactions and then annealed, or a DNA template with T7 RNA polymerase promoters on both ends of the template can be used to generate the two strands simultaneously (see Figure 40). This eliminates the need for a separate annealing step for transcripts <800 nt.

Simple Clean Up Procedure Yields dsRNA Ready for Use

The MEGAscript RNAi Kit provides all the reagents necessary to purify the resulting dsRNA. After the dsRNA is synthesized, it is digested with the included DNase I and single-strand specific RNase to degrade the DNA template and any residual ssRNA, respectively. The dsRNA is then purified away from proteins, free nucleotides, and nucleic acid degradation products using a quick glass fiber filter-based procedure.

The MEGAscript RNAi Kit is supplied with sufficient reagents to synthesize and purify 20 dsRNA molecules.

ORDERING INFORMATION		
Cat#	Description	Size
AM1626	MEGAscript® RNAi Kit	20 rxns

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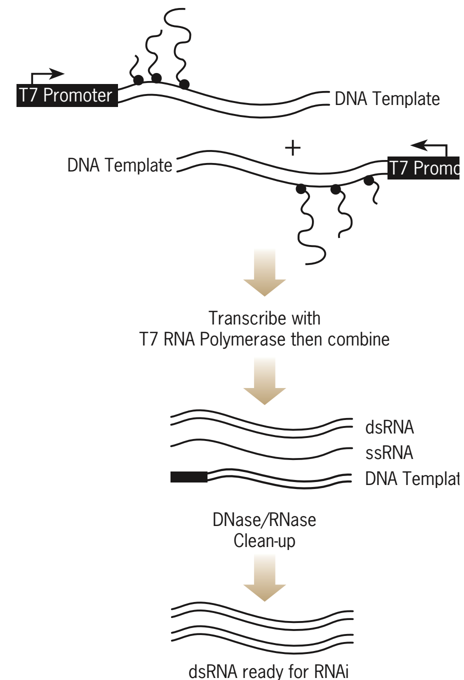

Figure 40. MEGAscript® RNAi Kit Protocol.

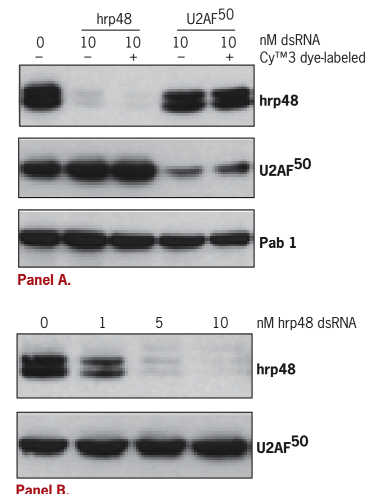

Figure 41. Silencing of *Drosophila* hrp48 and U2AF50 Protein by dsRNA. A. Specific Reduction of Expression Levels. 1 x 10^6 Schneider's *Drosophila melanogaster* L2 cells were grown in 6-well plates in serum-free medium and treated with 10 nM of dsRNA specific for either hrp48 or U2AF50. dsRNA was prepared with the MEGAscript® RNAi Kit and post-transcriptionally labeled with Cy™3 dye using the *Silencer*® siRNA Labeling Kit when indicated (+). Cells were harvested 72 hr post treatment and the silencing effect was analyzed by Western blot with anti-hrp48 (48 kDa, migrates as a doublet) and anti-U2AF50 (50 kDa) antibodies. An antibody directed against Pab 1 (lower panel) was used as a second negative control. **B.** Dose-sensitive Reduction of Protein Expression Levels. dsRNA-triggered silencing of hrp48 was analyzed as in Panel A using the indicated concentrations of hrp48 dsRNA made with the MEGAscript® RNAi Kit. Western analysis was also performed with the anti-U2AF50 (bottom panel) and Pab 1 (data not shown) antibodies as negative controls.

Silencer® *Drosophila* RNAi Library

For Genomewide RNAi Screens in *Drosophila*

- Ideal for high throughput RNAi screens in *Drosophila* cells
- Pre-made dsRNA library saves time and resources
- Sufficient quantities to perform dozens of screens in 384 well format

Application:
For performing genomewide RNAi screens in *Drosophila* cells

Delivery Method:
dsRNA can be delivered into *Drosophila* cells by addition to cultured cells, injection, or transfection, depending on cell type.

The *Silencer*® *Drosophila* RNAi Library, designed by Cenix BioScience, is the perfect tool for genome-wide RNAi screens in *Drosophila* cells. Each library includes 13,071 individual, double-stranded RNA (dsRNA) molecules representing the best annotated genes from the *Drosophila* genome. Purchase of a high quality ready-to-use dsRNA library means that you can spend your precious time on discovery rather than on reagent production, which can easily take 3 months or more of work even with elaborate liquid handling systems.

Each dsRNA in the *Silencer Drosophila* RNAi Library is prepared by PCR amplification of specific regions of the *Drosophila* genome followed by separate in vitro transcription reactions, annealing of the two strands, and clean up. The dsRNAs range in size from 300–800 base pairs—a size range previously shown to effectively elicit RNAi in *Drosophila*. After enzymatic synthesis, annealing, and purification, the dsRNA molecules are analyzed to ensure that they are of the proper size and yield. Typically fifteen micrograms of each dsRNA is provided in solution in 96 well plates, although other amounts are available.

ORDERING INFORMATION

Cat#	Description	Size
AM85000	*Silencer*® *Drosophila* RNAi Library	1 library

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MEGAscript® High Yield Transcription Kits

- Exclusive ultra high yield technology—synthesize more than 100 µg RNA in 2 hours or less
- Ideal for high throughput synthesis of dsRNA for RNAi experiments

The MEGAscript® High Yield Transcription Kits—the most frequently cited kits for in vitro transcription of RNA—use novel reaction conditions and Ambion's patented, high yield technology to synthesize 10 to 50 times the amount of RNA produced by conventional transcription reactions. Each DNA template is copied hundreds of times, so that milligram quantities of RNA can be produced from a single kit. A typical 20 µL T7 MEGAscript reaction with 1 µg of the pTRI-Xef-1alpha control template will yield over 100 µg of transcript. RNA synthesized from a MEGAscript reaction is suitable for many applications including induction of RNA interference in non-mammalian systems, array analysis, RNA vaccines, in vitro translation, microinjection, ribozyme studies, and RNA structural analysis. MEGAscript Kits are available in bulk quantities; contact us via email at **custom@ambion.com** for details.

ORDERING INFORMATION

Cat#	Description	Size
AM1330	MEGAscript® SP6 Kit	40 rxns
AM1333	MEGAscript® T7 Kit	25 rxns
AM1334	MEGAscript® T7 Kit	40 rxns
AM1338	MEGAscript® T3 Kit	40 rxns
AMB1334-5	5X MEGAscript® T7 Kit	200 rxns

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Bulk Reagents, Enzymes, & Kits

Ambion Custom

- All Ambion reagents, enzymes, and kits are available in bulk sizes
- Bulk packaging reduces costs and conserves time and resources
- Stringent quality control is guaranteed

Today's laboratory demands often require products and services that fall outside the range of standard kit configurations and sizes from typical life science reagent companies. Specialized protocols may quickly consume reagents in large volumes. For these situations, Ambion's kits, reagents, and enzymes are all available in bulk sizes that can save both time and resources.

To find out how Ambion can help meet your specific research requirements, email us at **custom@ambion.com**. R&D and production scientists are available to plan and design products specifically for your applications.

RNAqueous®-4PCR Kit

- Rapid, phenol-free total RNA isolation
- Includes novel reagent for rapid and safe removal of DNase and divalent cations
- Use with 100–10^7 cells per isolation

Application:
For generating long dsRNA designed to initiate RNAi in *C. elegans*, *Drosophila*, and other nonmammalian systems

The RNAqueous®-4PCR Kit provides RNA free of genomic DNA contamination from samples as small as 1 mg or 100 cells. The kit is especially suitable for RT-PCR applications and includes reagents to remove contaminating DNA from the isolated RNA. The kit is based on Ambion's RNAqueous technology of binding total RNA to glass fiber filters in a microfuge tube format. Briefly, the procedure consists of disrupting tissues or cells in a guanidinium-based lysis solution, followed by binding of the RNA to a glass fiber filter, washing the filter to remove contaminants, and then eluting the RNA in a small volume of elution solution.

MagMAX™-96 Total RNA Isolation Kit

- Reproducible total RNA isolation from 25 to 2 x 10^6 cells
- Higher yields than glass fiber filter methods
- Includes TURBO DNase™ Reagent to efficiently remove genomic DNA

Application:
96-well isolation of DNA-free total RNA for RT-PCR

Magnetic beads offer many benefits for isolating RNA from cultured cells. Ambion's MagMAX™ technology binds RNA more efficiently than glass fiber filter methods, resulting in higher RNA yields. Furthermore, the RNA yields are more consistent, both from experiment to experiment and over a broad range of sample sizes. Since only a small volume of magnetic beads is needed for high efficiency binding, the bound RNA can be eluted in just 20–50 µL of nuclease free water, resulting in highly concentrated RNA.

The MagMAX-96 Total RNA Isolation Kit is optimized for high throughput isolation of RNA from 25 cells to 2 x 10^6 cells, as well as small plant and mammalian tissue samples (up to 10 mg). TURBO DNase Reagent is included to improve genomic DNA removal, and the protocol is fully amenable to automation.

ORDERING INFORMATION

Cat#	Description	Size
AM1830	MagMAX™-96 Total RNA Isolation Kit	96 rxns
AM10050	96 well Magnetic-Ring Stand	1 each

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PARIS™ Kit

Protein and RNA Isolation System

- Isolate total RNA and protein from cultured cells or tissues
- No phenol extraction or alcohol precipitation
- Use with 100 to 10^7 cells

Application:
Isolation of total RNA and protein from the same sample

The PARIS Kit uses a fast, simple procedure to isolate both RNA and native protein from the same experimental sample, making the kit particularly useful for RNAi experiments. The resulting protein and RNA samples are suitable for various downstream applications, reducing time, cost, and variability between independent experimental samples.

In the PARIS procedure, tissue or cultured cells are first homogenized in Cell Disruption Buffer to prepare a total cell lysate. Total RNA is purified from part of the lysate using an RNA binding glass fiber filter. The other portion of the protein-containing lysate can be used directly for Western blotting, immunoprecipitation, functional assays, or two-dimensional gel electrophoresis.

ORDERING INFORMATION

Cat#	Description	Size
AM1921	PARIS™ Kit	50 purifications

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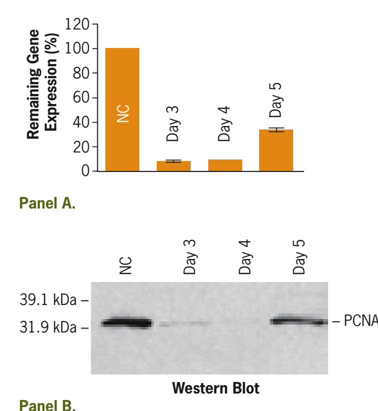

Figure 42. Timecourse of PCNA siRNA Effects on PCNA mRNA and Protein Levels Using the PARIS™ Kit. HeLa cells (30,000 cells/well in a 24-well plate) were transfected with 100 nM of an siRNA against PCNA or *Silencer*® Negative Control #1 (NC) using siPORT™ *Lipid* agent. Duplicate samples were harvested at 72 hours and each day for 5 days. The PARIS Kit was used to isolate both RNA and protein for either real-time RT-PCR **(Panel A)** or Western blot analysis **(Panel B)**. RNA was reverse transcribed using the RETROscript® Kit and target cDNA levels were measured by real-time PCR using SYBR® Green dye detection. Input cDNA in the different samples was normalized using real-time data for 18S rRNA. Data are shown relative to mRNA levels of cells transfected with negative control siRNA.

TaqMan® Gene Expression Assays

- Gene-specific TaqMan® probe and primer sets for quantitative gene expression studies in human, mouse, rat, *Rhesus*, *C. elegans*, *Drosophila*, and *Arabidopsis*
- Convenient single-tube format and 20X formulation
- Universal thermal cycling conditions

TaqMan Gene Expression Assays are a comprehensive collection of more than 600,000 predesigned primer and probe sets that let researchers quickly and easily perform quantitative gene expression studies on human, mouse, rat, *Rhesus*, *C. elegans*, *Drosophila*, and *Arabidopsis* genes. Each gene expression assay consists of a FAM™ dye-labeled TaqMan MGB probe and two PCR primers formulated into a 20X concentration in a single tube. Every assay is optimized to run under universal thermal cycling conditions with a final reaction concentration of 250 nM for the probe and 900 nM for each primer. This streamlined approach and comprehensive assay selection enable a convenient, standardized process for quantitative gene expression.

Approximately 40,000 TaqMan Gene Expression Assays for human, mouse, and rat are available as an inventoried, off-the-shelf product. This collection includes an average of 1 assay for each RefSeq mRNA transcript. Approximately 560,000 additional assays are available on a made-to-order basis covering most exon-exon junctions of mRNA transcripts for human, mouse, rat, *C. elegans*, *Drosophila*, and *Arabidopsis*.

Our extensive online catalog of TaqMan Gene Expression Assays is located at **www.appliedbiosystems.com**. Assays can be searched using a number of public ID numbers (including RefSeq ID, Entrez Gene ID, or Unigene ID), common gene names, symbols, or aliases, and functional categories and groups (such as kinase, cytokine, transcription factors). For a complete list of TaqMan Gene Expression Assays, visit **www.allgenes.com**.

Custom TaqMan® Gene Expression Assays

- Available for any species or organism
- Use the target sequence of your choice
- Provided in a convenient single-tube format

Custom TaqMan® Gene Expression Assays are available for any species, any splice variant, or any novel gene. Simply download our free File Builder Software to format and submit your target sequence. File Builder Software can be downloaded from **www.appliedbiosystems.com/filebuilder.** The software easily steps you through the ordering process, from selecting the assay size, formatting your target sequence to identify the location of the probe, and submitting your order via email.

All file submissions are done in a secure format. Your target sequences and the associated assays that are designed are kept confidential. With Custom TaqMan Gene Expression Assays, you benefit from Applied Biosystems proprietary software algorithms for primer and probe design, which enable you to obtain optimal assays for each target sequence. Assays are delivered in a single-tube, ready-to-use format, along with the primer and probe sequences designed from your submitted sequence.

Figure 43. TaqMan® Gene Expression Assays are delivered with a compact disc containing an electronic assay information file.

ORDERING INFORMATION

Description	Fill volumes	# of 20 µL reactions	DYE Labels	Universal Formulation	Delivery Time	Cat#
TaqMan® Gene Expression Assays						
Inventoried	250 µL, 20X	250	FAM™ DYE	Yes	3-5 days	4331182
Made-to-Order	360 µL, 20X	360	FAM™ DYE	Yes	5-10 days	4351372
Custom TaqMan® Gene Expression Assays	360 µL, 20X	360	FAM™ DYE	Yes	10-14 days	4331348
	750 µL, 20X	750				4332078
	967 µL, 60X	2,900				4332079

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Description	Fill volumes	# of 20 µL reactions	DYE Labels	Universal Formulation	Delivery Time	Cat#
TaqMan® Endogenous Controls						Various- see page 124
Not primer limited			FAM™ DYE	Yes		
Primer limited			VIC® DYE	Yes		
Custom TaqMan® Probes			FAM™ DYE	No	4-7 days	Various- see page 165
			VIC® DYE			
			TET™ DYE			
			NED™ DYE			

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Custom TaqMan® Probes and Primers

- Choice of dye labels, quenchers, and synthesis scale
- Available for any species or organism
- For use in quantitative gene expression, SNP genotyping, other allelic discrimination applications, and pathogen detection

When you know the exact sequences you need for your TaqMan® probes and primers, Applied Biosystems can synthesize them for you. As the market leader in real-time PCR, our high quality custom products can be used in all your real-time and end-point applications. These products offer you the ideal in flexibility if you prefer to optimize your own reaction formulation or if you simply prefer to buy in bulk.

ORDERING INFORMATION

	delivery	SIZE	CAT#
TaqMan® TAMRA™ Probes	4 to 5 days	6,000 pmol*	450025
	4 to 5 days	20,000 pmol*	450024
	4 to 5 days	50,000 pmol*	450003

TaqMan® Probes are available with a choice of 5' Fluorescent label: 6FAM™, VIC® or TET™ dyes* and 3' Quencher TAMRA™.

All probes are HPLC purified and sequence verified by mass spectrometry.

	delivery	SIZE	CAT#
TaqMan® MGB Probes	6 to 7 days	6,000 pmol*	4316034
	6 to 7 days	20,000 pmol*	4316033
	6 to 7 days	50,000 pmol*	4316032

TaqMan® MGB probes are available with a choice of 5' Fluorescent label: 6FAM™, VIC®, TET™*, or NED™ dyes** and a 3' minor groove binder/non-fluorescent quencher.

All probes are HPLC purified and sequence verified by mass spectrometry.

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* Please note that filter based instruments such as ABI PRISM® 7000 Sequence Detection System, Applied Biosystems 7300/7500/7500 Fast Real-Time PCR Systems are not supplied with calibration plates for TET™ dye. These instruments may be custom calibrated to use TET™ dye in a single-plex reaction, but TET™ dye should not be used in a multiplex reaction with either FAM™ or VIC® dyes, as the TET™ dye will not be distinguished by these instruments.

* * Please note that the Applied Biosystems 7500 Real-Time PCR System is optimized for use with NED™ dye labeled probes. Probes labeled with NED™ dye will give lower signal intensity on other real-time instrument systems than probes labeled with 6FAM™, VIC®, or TET™ dye. 3' label: MGBNFQ (Minor groove binder/Non-fluorescent quencher).

Gene Expression

TaqMan® Probe	Reaction Volume 50 µL (96-well plates)	Reaction Volume 20 µL (384-well plates)
pmoles	Number of Reactions	
6,000	480	1,200
20,000	1,600	4,000
50,000	4,000	10,000

For use on Applied Biosystems Real-Time PCR Systems.

Figure 44. TaqMan® Probe Usage Chart for Gene Expression. For Gene Expression, the minimum number of reactions obtained from our TaqMan probe products were calculated based on Universal Assay conditions, primer concentrations of 900 nM, and probe concentrations of 250 nM. The numbers are shown for 50 µL and 20 µL reaction volumes.

Western-*Star*™ Immunodetection System

Description

Western-*Star*™ Immunodetection System is a highly sensitive chemiluminescent immunodetection system that provides speed, flexibility, control of film exposure time and the ability to use alternative imaging systems. Western-*Star* system incorporates CDP-*Star* substrate for detection of secondary antibody-alkaline phosphatase conjugates.

Advantages

Western-*Star* system generates a high intensity chemiluminescent signal that persists from hours to days, depending on the membrane type. CDP-*Star*® substrate provides a five- to tenfold higher signal intensity than CSPD® substrate. Images can be generated immediately on X-ray or instant film to provide permanent, hard-copy results. In addition, the high signal intensity and long-lived signal generated with CDP-*Star* substrate provides an ideal quantitative detection system for chemiluminescent phosphor screen and CCD camera imaging systems.

Applications

Western-*Star* immunodetection sytem is used for highly sensitive immunoblot detection of proteins, biotinylated proteins (Aragaetal., 2000), and phosphoproteins in protein extracts from many sources, including cell cultures and tissues. These reagents are compatible with multiple types of membranes, including PVDF, nylon and nitrocellulose. Detection is performed directly with AP-labeled secondary antibodies, or with indirect detection of biotinylated antibodies or biotinylated proteins with a streptavidin-AP conjugate (Avidx-AP™ conjugate). Western-*Star* System has been used for confirmation of protein knockdown for siRNA gene expression regulation experiments [1].

Product Configuration

Western-*Star*™ System Standard Size

T1048 (WL10RS)
with Goat Anti-Rabbit IgG AP Conjugate
T1046 (WL10MS)
with Goat Anti-Mouse IgG+IgM AP Conjugate
Capacity: 30 membrane blots (10 cm x 10 cm)
Contents:
- 100 mL CDP-*Star*® 0.25 mM Ready-to-Use substrate
- Secondary antibody AP conjugate
- 30 g I-Block™ blocking reagent
- 5 mL Nitro-Block-II™ chemiluminescence enhancer
- 150 mL 10X Assay Buffer concentrate
- 30 development folders (14 cm x 19 cm)

ORDERING INFORMATION

Cat#	Description	Size
Western-*Star*™ SYSTEM		
T1046	with Goat Anti-Mouse IgG+IgM AP Conjugate	standard size
T1048	with Goat Anti-Rabbit IgG AP Conjugate	standard size
Western-*Star*™ System Accessories		
T2191	AP-Labeled Secondary Antibody Conjugates	100 µL
T2192	AP-Labeled Secondary Antibody Conjugates	100 µL
T2015	I-Block™ Blocking Reagent	30 g
T2234	Tropifluor™ PVDF Membrane (15 cm x 15 cm)—5 Membranes/Pack	1 Pack
T2258	Development Folders (14 cm x 19 cm)—30 Folders/Pack	1 Pack

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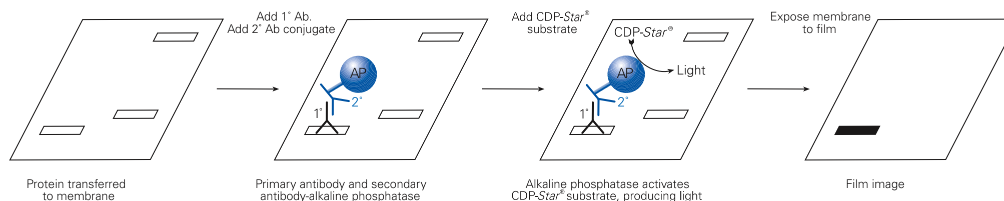

Figure 45. Immunodetection with Western-Star™ System.

RNaseZap® Solution and RNaseZap® Wipes

RNase Decontamination Solution and Wipes

RNase*Zap*® Solution removes RNase contamination from benchtops, pipettors, and equipment, protecting RNA from RNases that might otherwise be introduced into samples from these surfaces. RNase*Zap* Solution is a combination of three different chemicals that completely inactivates RNases immediately upon contact. Simply spray the surface with the solution and then rinse it thoroughly with nuclease-free water.

RNase*Zap* Wipes—towelettes pre-soaked with RNase*Zap* Solution—are particularly convenient for decontaminating pipettors and work surfaces.

ORDERING INFORMATION

Cat#	Description	Size
AM9780	RNase*Zap*® Solution	250 mL
AM9782	RNase*Zap*® Solution	6 x 250 mL
AM9786	RNase*Zap*® Wipes	1 container (100 sheets)
AM9788	RNase*Zap*® Wipes Refill	3 x 100 sheets

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Maintaining an RNase-free Lab

When using RNA in your lab, including siRNA, certain precautions should be taken:

1. Wear gloves throughout experiments to prevent contamination from RNases found on most human hands
2. Change gloves after touching skin, door knobs, and common surfaces
3. Dedicate a set of pipettors to be used solely for RNA work
4. Use tips and tubes that are tested and guaranteed to be RNase-free
5. Use RNase-free chemicals and reagents
6. Regularly decontaminate work surfaces with an RNase decontamination solution
7. Designate a "low-traffic" area of the lab that is away or shielded from air vents or open windows as an "RNase-free Zone".

For further details about handling RNA and keeping it intact, read *RNase Control: The Basics* at : **www.ambion.com/basics/rnasecontrol**

RNase-free Tips & Tubes

Tips and tubes are an easily overlooked source of RNase contamination. We consistently find a small percentage of tubes from other vendors (even those marketed as RNase-free), that cause RNA degradation. Simply autoclaving tips and tubes does not ensure elimination of RNases because these enzymes are very robust and will regain partial activity after cooling to room temperature.

Ambion provides RNase-free tips (including filter tips) of many sizes that are compatible with most commonly used pipettors. We also supply regular and non-stick microfuge tubes, which can be used for any molecular biology experiment. Each lot of RNase-free tubes and tips are subjected to exquisitely sensitive testing for RNase contamination and are guaranteed to be nuclease free.

ORDERING INFORMATION

Cat#	Description	Size
AM12225	Thin-walled, Frosted Lid, RNase-free PCR Tubes	1000 x 0.2 mL
AM12250	Thin-walled, Dome Cap, RNase-free PCR Tubes	1000 x 0.5 mL
AM12275	Thin-walled, Frosted Lid, RNase-free PCR Tubes	1000 x 0.5 mL
AM12300	RNase-free Microfuge Tubes	1000 x 0.5 mL
AM12350	Non-Stick RNase-free Microfuge Tubes	500 x 0.5 mL
AM12400	RNase-free Microfuge Tubes	500 x 1.5 mL
AM12425	RNase-free Microfuge Tubes	500 x 2.0 mL
AM12450	Non-Stick RNase-free Microfuge Tubes	250 x 1.5 mL
AM12475	Non-Stick RNase-free Microfuge Tubes	250 x 2.0 mL
AM12500	15 mL Conical Tubes (racked)	500 x 15 mL
AM12501	50 mL Conical Tubes (racked)	200 x 50 mL
AM12502	50 mL Conical Tubes (bagged)	250 x 50 mL
AM12635	Barrier (Filter) Tips, 10 µL size - Eppendorf®	Ten 8 x 12 racks
AM12640	Barrier (Filter) Tips, 10 µL size - Pipetman™	Ten 8 x 12 racks
AM12645	Barrier (Filter) Tips, 20 µL size	Ten 8 x 12 racks
AM12648	Barrier (Filter) Tips, 100 µL size	Ten 8 x 12 racks
AM12650	RNase-free Tips, 200 µL size	Ten 8 x 12 racks
AM12655	Barrier (Filter) Tips, 200 µL size	Ten 8 x 12 racks
AM12660	RNase-free Tips, 1000 µL size	Ten 100 ct racks
AM12665	Barrier (Filter) Tips, 1000 µL size	Ten 100 ct racks

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RNAi Information Resources

Since 2001, Ambion scientists have conducted thousands of siRNA experiments. The result is unrivalled expertise in siRNA design, synthesis, delivery and detection—as well as the broadest array of RNAi products available anywhere. Ambion is committed to sharing the information we have learned over the years. Below are some of the free resources Ambion makes available to researchers interested in RNAi and siRNA.

Technical Seminars

Ambion's scientists travel worldwide to provide technical seminars about RNAi and siRNA. Our seminars can be tailored to your interests and provide a great way to learn from and speak directly to Ambion scientists. If you are interested in hosting a seminar at your institution, contact us at **seminars@ambion.com**.

Technical Support from Highly Trained Scientists

Ambion employs scientists with advanced training and extensive experience to provide personalized support to researchers. Our RNAi and siRNA specialists provide help with:

- Experimental design
- Product selection
- Troubleshooting
- Ordering siRNAs and tracking existing siRNA orders

Contact us by telephone at the numbers printed on the back of this brochure, or via email at **techserv@ambion.com**.

Silencer® e-Newsletter

Ambion's *Silencer* e-mail Newsletter, published once a month, brings the latest information about the RNAi field right to your inbox. Sign up at **www.ambion.com/RNAi** to receive information about Ambion's newest products for RNAi related research. The newsletter also features tips and hints for improving results and brief summaries of articles of particular interest to those using RNAi in their research.

TechNotes Newsletter

Ambion's TechNotes Newsletter is published four times a year and includes informative articles on new RNA applications, "how-to" articles for improving results, and descriptions of Ambion's newest products. Several pages of each issue are devoted to RNAi and siRNA applications. Previous editions of TechNotes are available online at **www.ambion.com/techlib/tn**. Sign up for your free subscription to the print version of TechNotes at **www.ambion.com/info/litreq**.

Online RNA Interference Resource

Ambion's RNA Interference Resource provides a web portal to a wealth of information to help you harness the power of RNAi to study gene function.

- Ambion's database of siRNAs to all human, mouse, and rat genes, and siRNA design and delivery resources
- Interactive guides and articles for researchers new to RNAi
- Articles on RNAi research advances and tips for how to improve results
- Complete list of products for RNAi experiments
- Announcements about upcoming events
- Ambion webcasts and presentations
- The latest RNAi news from Ambion and around the world

Online siRNA Delivery Resource

Successful siRNA experiments require efficient and reproducible delivery of siRNAs into cells. Ambion's Online siRNA Delivery Resource can help you find the best siRNA delivery conditions for your system.

- Successful siRNA transfection and electroporation conditions for common cell types
- Articles on every aspect of siRNA delivery, including how to optimize experiments
- Peer reviewed publications from Ambion scientists
- Transfection, electroporation and delivery optimization kits and reagents

www.ambion.com/RNAi

www.ambion.com/siRNA/delivery

Appendix: siRNA Delivery Conditions by Cell Type

Below are some helpful tables of siRNA transfection and electroporation conditions. The online siRNA Delivery Resource is updated often. See **www.ambion.com/techlib/resources/delivery** for the latest information.

Optimized Transfection Conditions for Various Cell Types

Cell type	Reagent	Dish Size	Transfection Method	Number of cells (range)	Volume of Reagent (range)
22Rv1 (Human Prostate Carcinoma)	siPORT™ *NeoFX*™ Agent	96	Reverse	4,000–6,000	0.3–0.5 µL
293 (Human Kidney Epithelial, transformed)	siPORT™ *Amine* Agent	24	Forward	40,000–60,000	2 µL
293 (Human Kidney Epithelial, transformed)	siPORT™ *Amine* Agent	96	Reverse	6,000–8,000	0.4–0.6 µL
A549 (Human Lung Carcinoma)	siPORT™ *NeoFX*™ Agent	96	Reverse	4,000–5,000	0.4–0.6 µL
BJ (Human Foreskin Fibroblast)	siPORT™ *NeoFX*™ Agent	96	Reverse	1.000–2,000	0.6–1.2 µL
BT 549 (Human Mammary Ductal Carcinoma)	siPORT™ *NeoFX*™ Agent	96	Reverse	4,000–6,000	0.6–08 µL
CHO (Hamster Ovary Epithelial)	siPORT™ *Amine* Agent	24	Forward	30,000	2–3 µL
CHO (Hamster Ovary Epithelial)	siPORT™ *Amine* Agent	96	Reverse	6,000	0.15–0.3 µL
COS-7 (Monkey Kidney Fibroblast, transformed)	siPORT™ *NeoFX*™ Agent	96	Reverse	4,000–5,000	0.6–1.2 µL
HeLa (Human Cervix Adenocarcinoma)	siPORT™ *NeoFX*™ Agent	96	Reverse	4,000–5,000	0.15–0.3 µL
HeLa (Human Cervix Adenocarcinoma)	siPORT™ *NeoFX*™ Agent	6	Reverse	200,000	4.0 µL
HepG2 (Human Hepatocellular Carcinoma)	siPORT™ *NeoFX*™ Agent	96	Reverse	3,000–4,000	0.15–0.3 µL
HepG2 (Human Hepatocellular Carcinoma)	siPORT™ *NeoFX*™ Agent	6	Reverse	180,000	4.0 µL
HUVEC (Normal Human Umbilical Vein Endothelial Cells)	siPORT™ *NeoFX*™ Agent	96	Reverse	5,000–6,000	0.8–1.6 µL
MCF-12A (Human Mammary Gland, immortalized)	siPORT™ *NeoFX*™ Agent	96	Reverse	4,000–6,000	0.3–0.5
MCF-7 (Human Breast Adenocarcinoma)	siPORT™ *NeoFX*™ Agent	96	Reverse	5,000–6,000	0.6–1.2 µL
NIH/3T3 (Mouse Fibroblast)	siPORT™ *Amine* Agent	24	Forward	20,000–30,000	3–4 µL
NIH/3T3 (Mouse Fibroblast)	siPORT™ *Amine* Agent	96	Reverse	4,000–5,000	0.4–0.6 µL
SKNAS (Bone Marrow Neuroblastoma)	siPORT™ *NeoFX*™ Agent	96	Reverse	4,000–5,000	0.6–0.8 µL
TE 353.Sk (Human Skin Fibroblast, normal)	siPORT™ *NeoFX*™ Agent	96	Reverse	2,000–3,000	0.6–0.8 µL
TE 354.T (Human Basal Cell Carcinoma)	siPORT™ *NeoFX*™ Agent	96	Reverse	2,000–3,000	0.6–0.8 µL
UMR106 (Rat Osteosarcoma)	siPORT™ *NeoFX*™ Agent	96	Reverse	6,000–8,000	0.6–0.8 µL

These conditions are provided as a starting point for your convenience. For the best gene silencing results, we recommend that you test and preferably optimize these parameters. See pages 10–13 for more information on optimizing transfection conditions and selecting proper control siRNAs.

Optimized Conditions for Single Cuvette Electroporation with siPORT™ Electroporation Buffer

Cell Type	Voltage (Volts)	Pulse Length (microseconds)	Number of Pulses	Time between Pulses (seconds)	Number of Cells
BAEC (Bovine Aortic Endothelial Cells)	250	160	1–2	0.1	75,000
BAMEC (Bovine Adrenal Microvascular Endothelial Cells)	300	200	1	—	75,000
BAVSMC (Bovine Aortic Vascular Smooth Muscle Cells)	300	100	1–2	0.1	75,000
BLMVEC (Bovine Lung Microvascular Endothelial Cells)	300	140	1–2	0.1	75,000
hMSC (Human Mesenchymal Stem Cells)	700	90	2	5	75,000
HUVEC (Normal Human Umbilical Vein Endothelial Cells)	250	150	1	—	75,000
K562 (Human Lymphoblasts)	300–350	130	1–2	0.1	150,000
MEF (Mouse Embryo Fibroblasts)	350	150	2	0.1	75,000
NHA (Normal Human Astrocytes)	400	100	2–3	0.1	75,000
NHDF-neo (Normal Human Dermal Fibroblasts-neonatal)	900	70	2	5	75,000
PC-12 (Rat Pheochromocytoma)	450	200	1	—	75,000
Rat Astrocytes	300	90	2–3	0.1	75,000
RMSC (Rhesus Monkey Stem Cells)	450	120	2	0.1	75,000

Notes: Cells were resuspended in 75 µL of siPORT™ Electroporation Buffer and electroporation was performed with square wave type pulses in standard 1 mm electroporation cuvettes (Bio-Rad®).

Optimized Conditions for Electroporation with the siPORTer™-96 Electroporation Chamber and siPORT™ Electroporation Buffer

Cell Type	Voltage (Volts)	Pulse Length (microseconds)	Number of Pulses	Time between Pulses (seconds)	Number of Cells
hMSC (Human Mesenchymal Stem Cells)	800	200	1	—	25000
HUVEC (Human Umbilical Vein Endothelial Cells)	200	200	1 to 2	0.1	25000
Jurkat (Acute T-cell Leukemia)	200	250–350	1 to 2	0.1	200000
MEF (Mouse Embryo Fibroblsts)	500	200	1	—	25000
NHDF-neo (Normal Human Dermal Fibroblasts-neonatal)	600	400	1	—	25000
RAW264.7 (Mouse Macrophage)	300	400	1	—	25000
RMSC (Rhesus Monkey Stem Sells)	300	250	1 to 2	0.1	25000
RPTEC (Human Renal Proximal Tubule Cells)	350	350	1	—	25000
T cells (Human, Primary)	450	400	1	—	200000

Notes: Experiments were performed by electroporating eight identical samples simultaneously with the siPORTer™-96 Chamber using the above pulse conditions. One µg of siRNA was electroporated into the indicated number of cells in siPORT™ siRNA Electroporation Buffer at a final volume of 45 µL. These conditions are provided as a starting point for your convenience.

These conditions are provided as a starting point for your convenience. For the best gene silencing results, we recommend that you test and preferably optimize these parameters. See pages 10–13 for more information on optimizing transfection conditions and selecting proper control siRNAs.

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The RNA Interference Resource

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Ambion has been granted rights by the Massachusetts Institute of Technology to US Patent Applications 60/265232, 09/821832 and PCT/US01/10188, RNA Sequence-Specific Mediators of RNA Interference.

See pages 8-9 for ordering instructions and information on purity options.

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