# Recombinant Dicer Enzyme

## INSTRUCTION MANUAL

Catalog #240100
Revision #064001a

**For In Vitro Use Only**

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# Recombinant Dicer Enzyme

# Recombinant Dicer Enzyme

## MATERIALS PROVIDED

| Materials provided[a] | Quantity |
|---|---|
| Recombinant Dicer enzyme (0.5 U/µl) | 50 U |
| 5× Dicer reaction buffer[b] | 200 µl |

[a] Kit contains enough reagents for 50 dsRNA digestion reactions.

[b] See *Preparation of Reagents*.

## STORAGE CONDITIONS

**All Components:** –20°C

## ADDITIONAL MATERIALS REQUIRED

| Additional Materials | Suggested Products |
|---|---|
| DNA polymerase | Herculase® Enhanced DNA Polymerase (Stratagene; Catalog #600260, 600262, or 600264)[†] |
| dNTPs | — |
| PCR tubes | — |
| temperature cycler | — |
| PCR purification reagents | StrataPrep® PCR Purification Kit (Stratagene; Catalog #400771 or 400773)[††] |
| RNA transcription kit | RNAMaxx™ High Yield Transcription Kit (Stratagene; Catalog #200339) |
| lithium chloride precipitation solution[§] | — |
| siRNA purification reagents | • Microcon® YM-100 Centrifugal Filter Unit (Millipore Corporation; Catalog #42412)<br>• MicroSpin G-25 Columns (Amersham Biosciences; Catalog #27-5325-01) |
| siRNA transfection reagents | GeneEraser™ siRNA Transfection Reagent (Stratagene; Catalog #204150) |
| transfection tubes | 5-ml BD Falcon polystyrene round bottom tubes (BD Biosciences catalog #352054) |
| media for cell growth | — |
| media for transfection | — |
| RNase-free DNase | RNase-free DNase (Stratagene; Catalog #600031 or 600032) |

[†,††] See *Endnotes*.

[§] See *Preparation of Reagents*.

Revision #064001a

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# INTRODUCTION

RNA interference (RNAi), also known as gene silencing, is a cellular process responsible for the degradation of a specific mRNA species by the introduction of homologous double-stranded RNA (dsRNA) molecules into the cell. The RNAi process involves the in vivo cleavage of exogenous dsRNA into 21–26 nucleotide small interfering dsRNA molecules (siRNAs) by the Dicer protein, an endogenous ribonuclease. The siRNAs assemble with an RNA–protein complex that binds and degrades the complementary mRNA transcript. This mRNA-specific degradation is an effective means of gene suppression and is therefore used to study gene function in many eukaryotic cell types, including plant, yeast, insect, and mammalian cells.[1–3]

Although the introduction of long dsRNAs into non-mammalian cells (e.g., insect or plant cells) has been proven to successfully suppress specific genes, the transfection of long dsRNAs into mammalian cells triggers a strong antiviral response, resulting in nonspecific inhibition of protein synthesis.[4,5] To overcome this problem, recent studies have shown that introducing double-stranded RNA shorter than 30 bp into mammalian cells results in the suppression of specific genes without eliciting the antiviral response observed with long dsRNAs.[6]

Different methods exist for the design and introduction of siRNA into mammalian cells, including siRNA oligonucleotide design and synthesis followed by either transfection of the siRNA oligos or of an siRNA hairpin-expressing vector into mammalian cells.[6,7] These experiments typically require the design of a single 19–29 nucleotide siRNA sequence homologous to a region of the target gene sequence. In order to identify an siRNA sequence that optimally suppresses a target gene, siRNA sequences homologous to different target gene regions must be designed, synthesized, and tested, which can be time-consuming and expensive.

Stratagene offers a more cost-effective and rapid alternative to this method, which involves the in vitro digestion of a long target-specific dsRNA molecule into short siRNAs by the recombinant Dicer enzyme,[8] followed by siRNA transfection into mammalian cells (see Figure 1). This method eliminates the difficulty of selecting a single siRNA sequence that optimally suppresses the target gene, which often requires an empirical approach. Instead, Dicer-mediated cleavage generates a comprehensive pool of siRNA molecules representing the entire target region.

The recombinant Dicer enzyme cleaves greater than 95% of a 500-bp dsRNA template into a pool of 21–23 bp siRNA molecules within 18 hours when used according to the optimal reaction conditions provided. Dicer cleaves dsRNA without apparent sequence preference,[9] and the siRNA molecules generated by Dicer are suitable for gene-specific suppression by RNAi in mammalian cells.[8]

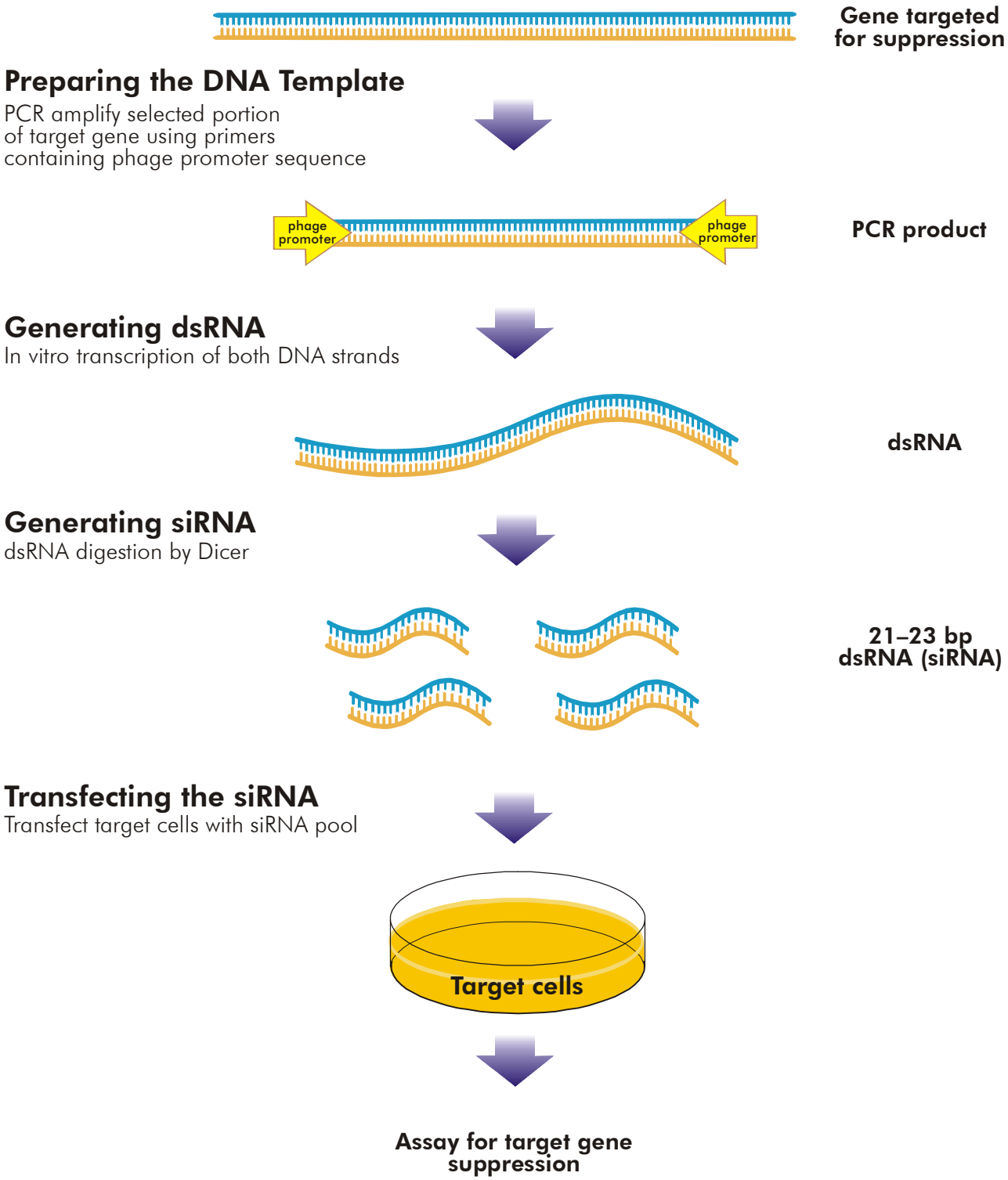



**FIGURE 1** siRNA generation and target gene suppression overview.

**Note** *The protocols provided give instructions for performing all steps of siRNA generation and transfection, including preparing the DNA template for transcription, generating dsRNA transcripts, cleaving the dsRNA into siRNA using Dicer, and siRNA purification and transfection into mammalian cells. For instructions on using the recombinant Dicer enzyme only, see* Digesting the dsRNA Using Dicer *in* Generating siRNA.

# PREPARING THE DNA TEMPLATE

## Selecting the Target Sequence and Size

When selecting the region of the gene sequence targeted, avoid selecting target regions near the start codon or in untranslated regions (UTRs), as these regions in the target gene may not be accessible by the siRNA molecule due to bound regulatory proteins or complicated structures.[10]

The optimal size of the target dsRNA molecule that will be digested by Dicer and that will suppress the target gene is approximately 500–750 bp in length. While smaller target dsRNA sizes are effectively cleaved by Dicer, Stratagene has found that transcription and purification yields decrease significantly.

## Generating the Template for Transcription

The recombinant Dicer enzyme cleaves dsRNA molecules that are prepared by in vitro transcription of DNA encoding the target region. In order to generate a dsRNA molecule, two complementary single-stranded DNA (ssDNA) molecules must be transcribed and annealed. While many options exist for generating two complementary RNA transcripts, Stratagene recommends performing one transcription reaction from a single dsDNA template that has been prepared by PCR amplification of the target region with PCR primers containing the T7 phage promoter sequence.

If both RNA strands are produced in the same transcription reaction using the same phage promoter, the ratio of sense and antisense transcripts will be equal. An annealing step is generally not required since the two complementary transcripts form duplexes during the reaction. In addition, RNase contamination is less of a concern since the transcription reaction will not contain ssRNA molecules.

Alternatively, using linearized plasmid DNA as a template in the transcription reaction is an option, however this method is often more time-consuming. For example, if two different phage promoters are used to generate each of the two complementary transcripts, two different transcription reactions are required. In this case, the product yield of each reaction must be determined separately following transcription, and the appropriate amounts of each strand must be annealed to form the dsRNA duplex. In addition, care must be taken to prevent RNase contamination of the transcription and annealing reactions since they involve ssRNA transcripts.

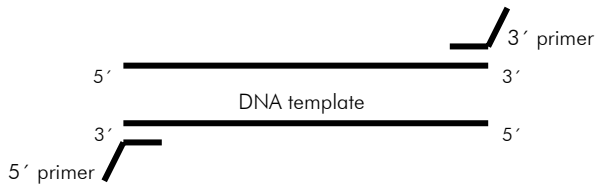
## PCR-Amplifying the Target Sequence

### Designing the PCR Primers

The T7 phage promoter typically generates the highest yields of RNA, when compared to the T3 and SP6 phage promoters. The T7 promoter is minimally a 19 base pair sequence (5′ TAATACGACTCACTATAGG 3′) that directs T7 RNA polymerase transcription initiation with high specificity. The in vitro transcription protocol provided uses T7 RNA polymerase to produce double-stranded RNA from a double-stranded DNA template containing a T7 promoter on each strand.

The DNA template in the transcription reaction must contain a phage promoter sequence on the 5′ end of the sense strand and the same phage promoter sequence on the 5′ end of the antisense strand in the reverse direction. It is therefore recommended that the T7 promoter sequence be added to the 5′ end of each PCR primer used to amplify the target sequence. A GC-rich stabilizer sequence (5′ GCG 3′) should be present on the 5′ end of each T7 promoter sequence, acting as a clamp for the transcription product duplexes (see Figure 2).

**A.**




**B.**

**5′ primer** 5′GCGTAATACGACTCACTATAGGNNNNNNNNNNNNNNNNNNN 3′

stabilizer T7 promoter sequence 5′ end of sense strand

**3′ primer** 5′GCGTAATACGACTCACTATAGGNNNNNNNNNNNNNNNNNNN 3′

stabilizer T7 promoter sequence 5′ end of antisense strand

**FIGURE 2** PCR primer design. (A) Orientation of the 5′ and 3′ PCR primers to the target portion of the template. (B) Recommended PCR primer sequences, including the stabilizer, T7 promoter sequence, and approximately 20 bases that are complementary to the 3′ ends of the target sequence sense and antisense strands.

## Preparing and Cycling the PCR Mixture

***Note*** *The following PCR protocol gives general reaction conditions for amplifying the target sequence from cloned DNA template. Stratagene recommends using a robust, high-fidelity DNA polymerase, such as Herculase® enhanced DNA polymerase (Catalog #600260, 600262, or 600264).*

1. Add the following components to a PCR reaction tube *in order* while mixing gently. The recipe listed in Table I is for one reaction and must be adjusted for multiple samples. The final volume of each sample reaction is 50 µl.

**TABLE I**

**Reaction Mixture for PCR Amplification of the Target Region from a Cloned Template**

| Component | Amount per reaction |
|---|---|
| Distilled water ($dH_2O$) | *X* µl to final volume of 50 µl |
| 10× PCR reaction buffer | 5.0 µl |
| dNTPs (200 µM each dNTP) | *X* µl |
| DNA template (1–50 ng) | *X* µl |
| Primer #1 (100 ng) | *X* µl |
| Primer #2 (100 ng) | *X* µl |
| DNA polymerase | *X* µl |
| Total reaction volume | 50 µl |

2. Immediately before thermal cycling, aliquot the reaction mixture into a sterile thin-wall PCR tube or a standard 0.5-ml microcentrifuge tube.

3. If the temperature cycler is not equipped with a heated cover, overlay the reaction with ~50 µl of DNase-, RNase-, and protease-free mineral oil (Sigma, St. Louis, Missouri).

4. Perform PCR using the optimized cycling conditions in Table II.

**TABLE II**

**PCR Cycling Parameters**

| Segment | Cycles | Temperature | Duration |
|---|---|---|---|
| 1 | 1 | 95°C | 3 minutes |
| 2 | 20 | 95°C | 30 seconds |
| | | 55°C | 30 seconds |
| | | 72°C | 1 minute/kb |
| 3 | 1 | 72°C | 5 minutes |

5. Analyze the PCR amplification products on a 0.7–1.0% (w/v) agarose gel.

6. Purify the PCR product using a suitable method for use in the RNA transcription reaction.

**Note** *Stratagene's StrataPrep® PCR Purification Kit (Catalog #400771 or 400773) is available for purifying the PCR product.*

# GENERATING DSRNA

## RNA Transcription Reaction

**Note** *Stratagene's RNAMaxx™ high yield transcription kit (Catalog #200339) is available for generating large quantities of RNA from the T7 phage promoter. The following transcription protocol uses components provided by the RNAMaxx transcription kit.*

1. The following amounts are for a typical 25-µl transcription reaction. Keep the ribonucleotides, T7 RNA polymerase, yeast inorganic pyrophosphatase and RNase block on ice and the remaining components at room temperature while preparing the reaction mixture. Add the components *in order* while mixing gently.

| Component | Amount per reaction |
|---|---|
| 5× RNAMaxx™ transcription buffer | 5 µl |
| Distilled water ($dH_2O$) | *X* µl |
| 100 mM rUTP | 1 µl |
| 100 mM rATP | 1 µl |
| 100 mM rGTP | 1 µl |
| 100 mM rCTP | 1 µl |
| PCR-generated DNA template (1 µg) | *X* µl |
| 0.75 M DTT | 1 µl |
| 0.75 U/µl of yeast inorganic pyrophosphatase | 0.5 µl |
| RNase block | 1 µl |
| 200 U/µl T7 RNA polymerase | 1 µl |
| Total reaction volume: | 25 µl |

2. Incubate the reaction at 37°C for 2 hours.

3. Purify and analyze the reaction products immediately (see *Purifying* and *Analyzing the RNA Transcription Products*) or store the reaction at –20°C until analysis.

## Purifying the RNA Transcription Products

**Note** *The following protocol purifies the double-stranded nucleic acid molecules using a lithium chloride precipitation method. This method is not recommended for transcripts smaller than 300 bp.*

1. Add 25 µl of sterile water and 25 µl of lithium chloride precipitation solution (see *Preparation of Reagents*) to the transcription reaction tube from above.

2. Briefly vortex the mixture. Incubate the tube at –20°C for at least 30 minutes.

3. Centrifuge the tube at 4°C for 15 minutes at 10,000 × g to pellet the dsRNA.

4. Carefully discard the supernatant. Wash the pellet with 1 ml of 70% ethanol.

5. Centrifuge at maximum speed for 10 minutes.

6. Remove the ethanol and allow the pellet to air dry. Resuspend the dsRNA in nuclease-free water.

7. Analyze the reaction products immediately (see *Analyzing the RNA Transcription Products*) or store the reaction at –20°C until analysis.

## Analyzing the RNA Transcription Products

### Quantitating RNA Yield Using Spectrophotometry

The approximate RNA yield can be determined using UV spectrophotometry at 260 nm. The conversion factor for RNA is 0.040 µg/µl per $OD_{260}$ unit. For an $A_{260}$ reading of 0.20 of an RNA sample diluted 1:100, calculate the concentration as follows:

$$\text{Concentration} = A_{260} \times \text{dilution factor} \times \text{conversion factor}$$

***Example*** $0.20 \times 500/5 \times 0.040\ \mu g/\mu l = 0.8\ \mu g/\mu l$

Calculate the yield of RNA by multiplying the volume in microliters by the concentration. If the sample volume in this example is 100 µl, the RNA yield is 80 µg.

### Quantitating RNA Yield Using RiboGreen®

The use of a fluorescence-based system provides a more sensitive RNA quantitation method. Using this system, a fluorescence microplate reader or fluorometer and an RNA-specific intercalating fluorescent dye (i.e. RiboGreen® RNA Quantitation Kit, Molecular Probes, Inc.) are required. The RNA concentration of a sample is determined by comparing the sample absorbance to a standard curve of known sample concentrations. Refer to the manufacturer's instructions for fluorescence-based RNA quantitation.

### Determining dsRNA Size Using Agarose Gel Electrophoresis

The dsRNA transcript size may be checked for accuracy before proceeding to the Dicer digestion reaction by electrophoresis of the transcription products on a 1% agarose gel.

**Visualization using ethidium bromide:** If the gel loading buffer used contains ethidium bromide, examine the gel with UV illumination.

***Note*** *Wear UV-protective safety glasses or a full safety mask to prevent UV damage to the face and skin.*

**Visualization using SYBR® Green II:** Incubate the gel in 50 ml of water or TE buffer (see *Preparation of Reagents*) containing 5 µl of SYBR® Green II gel stain at room temperature for 10 minutes with gentle rocking. The stained RNA can be visualized on an imaging instrument using SYBR Green filters.

***Note*** *Additionally, Stratagene recommends analyzing the dsRNA transcription products following the Dicer reaction, when the dsRNA product size and intensity can be compared side-by-side with the siRNA product size and intensity (see* Determining dsRNA Versus siRNA Sizes and Intensities Using PolyAcrylamide Gel Electrophoresis (PAGE) *in* Analyzing the siRNA*).*

## Digesting the dsRNA Using Dicer

Each reaction containing 1 µg of dsRNA will yield approximately 500 ng of siRNA following purification.

1. Add the following components in order while mixing gently.

| Component | Amount per reaction |
|---|---|
| 5× Dicer reaction buffer[a] | 2 µl |
| Recombinant Dicer enzyme (0.5 U/µl) | 2 µl |
| dsRNA (1 µg) | *X* µl |
| Distilled water ($dH_2O$) | *X* µl |
| Total reaction volume: | 10 µl |

[a] The 5× Dicer reaction buffer intentionally does not contain ATP since the recombinant Dicer enzyme provided is not ATP-dependent.[11,12]

**Note** *Dicer activity is significantly reduced if the sodium chloride concentration of 250 mM is altered. During the Dicer reaction, NaCl is contributed by both the enzyme and reaction buffers. Therefore, it is important that the ratio of buffer and enzyme (1:1) be maintained during the reaction.*

2. Incubate the reaction at 37°C for 18–20 hours.

3. Purify the reaction products immediately or store the reaction at –20°C until analysis.

## Purifying the siRNA

The siRNA purification strategy involves two separate purification steps to remove salts, unincorporated nucleotides, and undigested dsRNA transcripts. Following the siRNA purification methods, approximately 50–60% of the siRNA from the digestion reaction will be retained.

**Note** *Up to four Dicer reactions can be combined and purified simultaneously.*

### Removing Salts and Unincorporated Nucleotides

**Note** *The following nucleotide purification protocol uses the MicroSpin G-25 column (Amersham Biosciences; Catalog #27-5325-01), which removes salts and unincorporated nucleotides from the reaction products. The instructions provided differ slightly from the manufacturer's recommendations; Stratagene has obtained greater purity by performing an additional wash step.*

1. Prepare the column by gently vortexing the resin.

2. Loosen the cap one-quarter turn and snap off the bottom closure. Place the column in a 1.5-ml microcentrifuge tube.

3. Spin the column at 735 × g for 1 minute to remove the preservative solution. Discard the eluent.

4. Wash the resin by adding 100 µl of water to the column and spin for 1 minute at 735 × g. Discard the eluent. Repeat the wash step once.

5. Place the column in a fresh 1.5-ml microcentrifuge tube. Increase the volume of the Dicer reaction to 100 µl by adding sterile water and mix by gently flicking the tube. Gently apply the diluted Dicer reaction products to the center of the compacted column's resin bed without disturbing the resin.

6. Spin the column for 2 minutes at 735 × g. Retain the filtrate.

### Removing Undigested dsRNA

***Note*** *The following nucleotide purification protocol uses the Microcon® YM-100 column (Millipore Corporation; Catalog #42412), which removes undigested dsRNA from the reaction products.*

1. Carefully pipette the filtrate from step 6 above onto the new column filter.
2. Spin the column for 12 minutes at 500 × g. Retain the filtrate, which contains the purified 21–23 bp dsRNA molecules.
3. The purified dsRNA can be stored at –20°C for one week or at –80°C for up to 6 months.

## Analyzing the siRNA

### Quantitating siRNA Yield Using Spectrophotometry

The approximate RNA yield can be determined using UV spectrophotometry at 260 nm. The conversion factor for RNA is 0.032 µg/µl per $OD_{260}$ unit. For an $A_{260}$ reading of 0.20 of an RNA sample diluted 1:100, calculate the concentration as follows:

$$\text{Concentration} = A_{260} \times \text{dilution factor} \times \text{conversion factor}$$

***Example*** $0.20 \times 500/5 \times 0.032\ \mu g/\mu l = 0.64\ \mu g/\mu l$

Calculate the yield of RNA by multiplying the volume in microliters by the concentration. If the sample volume in this example is 100 µl, the RNA yield is 64 µg.

### Quantitating RNA Yield Using RiboGreen®

The use of a fluorescence-based system provides a more sensitive RNA quantitation method. Using this system, a fluorescence microplate reader or fluorometer and an RNA-specific intercalating fluorescent dye (i.e. RiboGreen RNA Quantitation Kit, Molecular Probes, Inc.) are required. The RNA concentration of a sample is determined by comparing the sample absorbance to a standard curve of known sample concentrations. Refer to the manufacturer's instructions for fluorescence-based RNA quantitation.

### Determining dsRNA Versus siRNA Sizes and Intensities Using PolyAcrylamide Gel Electrophoresis (PAGE)

Stratagene recommends that the dsRNA transcription reaction products be analyzed in an adjacent lane to the siRNA Dicer digestion reaction products on a 4–20% TBE polyacrylamide gel. If equal amounts of dsRNA are loaded on the gel, this analysis will reveal product sizes and differences in product intensities, indicating that the long dsRNA was successfully cleaved by Dicer (see Figure 3).

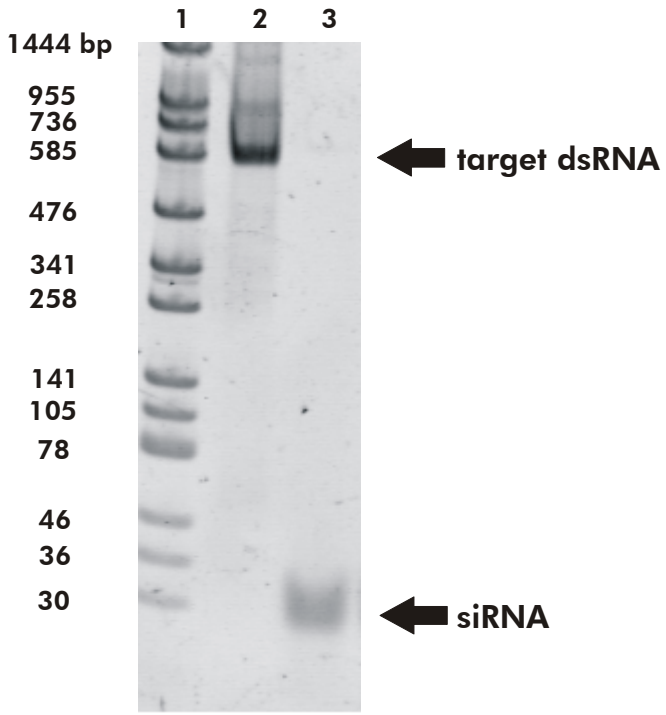



**FIGURE 3** A 4–20% TBE polyacrylamide gel comparing the dsRNA transcription products and the Dicer-mediated cleavage products. RNA transcription, Dicer digestion, and purification reactions were performed according to the *Recombinant Dicer Enzyme Instruction Manual*. **Lane 1**, molecular weight marker; **Lane 2**, dsRNA transcription product prior to digestion by Dicer (500 bp); **Lane 3**, siRNA product after digestion by Dicer.

## TRANSFECTING THE SIRNA

Target gene suppression may vary depending upon the transfection efficiency, the expression level of the target gene, and the mRNA stability. The efficiency of transfection will vary depending on the method of transfection, siRNA quality, and the host cell line used. Varying the amount of siRNA may be required to identify optimal target gene suppression conditions. In most cases, suppression of the target gene should be detectable 24–72 hours after transfection. The length of time is dependent upon mRNA and protein stability and the method used to detect suppression. A time course is recommended to identify the optimal time to detect gene suppression. Perform the appropriate assays to determine target gene suppression levels.

**Note** *Stratagene's GeneEraser™ siRNA transfection reagent (Stratagene Catalog #204150 and 204152) provides a simple method for high-efficiency siRNA transfection of a wide variety of cell types.*

# TROUBLESHOOTING

| Observation | Suggestion |
|---|---|
| Failed PCR reaction | Each PCR primer contains a 22-bp extension on the 5′ end that may prevent the annealing of the primers to the template. Redesign the primers by changing or extending the gene-homologous portions. |
| | Optimize the PCR conditions. |
| Low RNA yield following the transcription reaction | The DNA template must be pure. DNA should be purified with multiple phenol–chloroform extractions until the interface is clear. Cesium chloride banding is the preferred method of purifying DNA. Check the $OD_{260/280}$ ratio (expect 1.8–2.0). When generating the DNA template using PCR, the StrataPrep PCR purification kit may be used to purify the PCR product. |
| | Excessive NaCl from the input DNA template may inhibit the T7 RNA polymerase. Make sure all NaCl is removed from the DNA preparation. A 70% (v/v) ethanol wash of the DNA pellet is recommended after precipitation. When generating the DNA template using PCR, the StrataPrep PCR purification kit may be used to remove the excess salt. |
| The transcription reaction products give a smear when visualized on an agarose gel | Perform the following reaction to anneal the two RNA transcripts. (Ensure that the samples are RNase-free.):<br>• Following the 2-hour transcription incubation, add 1 µl of 10 U/µl RNase-free DNase. Incubate at 37°C for 30 minutes.<br>• Heat the mixture to 95°C and incubate for 5 minutes.<br>• Cool the mixture slowly (for a minimum of 1 hour) until the reaction reaches room temperature. |
| No siRNA product or low siRNA yield | The dsRNA quality may be poor. |
| | Not enough dsRNA was used in the Dicer reaction. Ensure that 1 unit of Dicer is used for every 1 µg of RNA in the Dicer reaction mixture. |
| | Do not use excess Dicer. Ensure that 1 unit of Dicer is used for every 1 µg of RNA in the Dicer reaction mixture. |
| | If scaling-up the Dicer reaction, maintain the ratio of recombinant Dicer enzyme and 5× Dicer reaction buffer. |
| The transfection efficiency is low | Use an siRNA transfection protocol that has been optimized for the cell line used. |
| | Verify the siRNA concentration. |
| The incidence of cell toxicity is high | Perform a dose-response curve varying the amount of transfection reagent while maintaining the amount of siRNA to determine if too much reagent is used; alternatively, execute a dose-response curve varying the amount of siRNA while maintaining the amount of transfection reagent to determine if too much siRNA is used. |
| | Ensure even distribution of the transfection mixture to all of the cells by rocking the dish back and forth and side to side. |
| | Transfection reagents may be toxic to the cell line. Use a different method of transfection. |
| | Suppression of the target gene may affect cell viability. |

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| Observation | Suggestion |
|---|---|
| Little or no suppression of the target gene | Increase or decrease the length of time between siRNA transfection and detection of gene suppression. Generally, target gene suppression should be detectable 24–72 hours following transfection. A time course is recommended to identify the optimal time to detect target gene suppression. |
| | Increase or decrease the amount of siRNA transfected. An siRNA concentration titration is recommended to identify the optimal siRNA concentration required to detect target gene suppression. |
| | Verify that target protein detection reagents are functional. |

## PREPARATION OF REAGENTS

### 5× Dicer Reaction Buffer

100 mM Tris-HCl (pH 8.0)
750 mM NaCl
12.5 mM $MgCl_2$

### TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

### Lithium Chloride Precipitation Solution

7.5 M LiCl
50 mM EDTA
Bring to a final pH of 7.0 with NaOH

## REFERENCES


1. Hannon, G. J. (2002) *Nature* 418:244–51.
2. Elbashir, S. M., Lendeckel, W. and Tuschl, T. (2001) *Genes Dev* 15:188–200.
3. Martinez, J., Patkaniowska, A., Urlaub, H., Luhrmann, R. and Tuschl, T. (2002) *Cell* 110:563–74.
4. Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H. and Schreiber, R. D. (1998) *Annu Rev Biochem* 67:227–64.
5. Williams, B. R. (1997) *Biochem Soc Trans* 25:509–13.
6. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. *et al.* (2001) *Nature* 411:494–498.
7. Paddison, P. J., Caudy, A. A., Bernstein, E., Hannon, G. J. and Conklin, D. S. (2002) *Genes Dev* 16(8):948–958.
8. Myers, J. W., Jones, J. T., Meyer, T. and Ferrell, J. E. (2003) *Nat Biotechnol* 21:324–8.
9. Bernstein, E., Caudy, A. A., Hammond, S. M. and Hannon, G. J. (2001) *Nature* 409:363–366.
10. Elbashir, S. M., Harborth, J., Weber, K. and Tuschl, T. (2002) *Methods* 26:199–213.
11. Nykanen, A., Haley, B. and Zamore, P. D. (2001) *Cell* 107:309–21.
12. Billy, E., Brondani, V., Zhang, H., Muller, U. and Filipowicz, W. (2002) *Embo J* 21:5875–85.


## ENDNOTES

† Purchase of this product is accompanied by a license to use it in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., an authorized thermal cycler.

†† Purchase of this PCR-related product does not convey any rights under the PCR patents owned by Roche Molecular Systems. A license to use the PCR process accompanies the purchase of certain reagents from Stratagene when used in conjunction with an Authorized Thermal Cycler.



## MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on Stratagene's website at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE®

# Recombinant Dicer Enzyme

Catalog #240100

## QUICK-REFERENCE PROTOCOL

***Note*** *For a complete set of protocols that give instructions for preparing the DNA template for transcription, generating dsRNA transcripts, cleaving the dsRNA into siRNA using Dicer, and siRNA purification and transfection into mammalian cells, see the* Recombinant Dicer Enzyme Instruction Manual.

### Generating siRNA Using Dicer

Each reaction containing 1 µg of dsRNA will yield approximately 500 ng of siRNA following purification.

- Add the following components in order while mixing gently.

| Component | Amount per reaction |
|---|---|
| 5× Dicer reaction buffer[a] | 2 µl |
| Recombinant Dicer enzyme (0.5 U/µl) | 2 µl |
| dsRNA (1µg) | *X* µl |
| Distilled water ($dH_2O$) | *X* µl |
| Total reaction volume: | 10 µl |

[a] The 5× Dicer reaction buffer intentionally does not contain ATP since the recombinant Dicer enzyme provided is not ATP-dependent.[11,12]

***Note*** *Dicer activity is significantly reduced if the sodium chloride concentration of 250 mM is altered. During the Dicer reaction, NaCl is contributed by both the enzyme and reaction buffers. Therefore, it is important that the ratio of buffer and enzyme (1:1) be maintained during the reaction.*

- Incubate the reaction at 37°C for 18–20 hours.
- Purify the reaction products immediately or store the reaction at –20°C until analysis.