

dsDNA Conversion Kit for RiboMultiplier™ sRNA

Cat. No. RMD80625

The dsDNA Conversion Kit for RiboMultiplier sRNA converts amplified sense-RNA (sRNA) produced by the RiboMultiplier Sense-RNA Amplification Kit (Epicentre; patent pending) to full-length double-stranded DNA (dsDNA). Due to the nature of the dsDNA synthesis primer, only sRNA produced by the RiboMultiplier Sense-RNA Amplification Kit can be converted to full-length dsDNA using this kit. Each dsDNA Conversion Kit reaction converts 5-20 µg of sRNA into dsDNA in about 2.5 hours.

Important! This kit should only be used for converting amplified sRNA produced by the RiboMultiplier Sense-RNA Amplification Kit to dsDNA.

Storage: Store the kit at -20°C in a freezer without a defrost cycle.

Additional Required Reagents and Equipment:

Thermocycler

Microcentrifuge

DNA Purification columns such as: QIAquick® PCR Purification Kit (Qiagen; Cat. No. 28104)

Kit Contents

The kit components are supplied in tubes with colored caps for easier identification.

Component Name	Tube Label	25 Reactions
Red-Cap Tubes		
1 st -Strand cDNA Primer	1 st -Strand cDNA Primer	15 µl
StarScript Reverse Transcription Buffer	StarScript RT Buffer	125 µl
RiboGuard RNase Inhibitor (16 U/µl)	RiboGuard RNase Inhibitor	30 µl
dNTP PreMix	dNTP PreMix	60 µl
StarScript Reverse Transcriptase (35 U/µl)	StarScript RT	15 µl
cDNA Finishing Solution	cDNA Finishing Solution	15 µl
Blue-Cap Tubes		
2 nd -Strand dsDNA Primer	2 nd -Strand dsDNA Primer	30 µl
DNA Polymerase	DNA Polymerase	30 µl
100 mM Dithiothreitol (DTT)	DTT	90 µl
Colorless-Cap Tubes		
RNase-Free Water	RNase-Free Water	500 µl

Performance Specifications and Quality Control

The dsDNA Conversion Kit for RiboMultiplier sRNA is function-tested in a control reaction. The kit must produce at least 2.5 µg of dsDNA from 10 µg of RiboMultiplier sRNA. dsDNA status is confirmed by resistance to digestion with Mung Bean Nuclease, a single-strand-specific nuclease.

Applications of dsDNA produced from RiboMultiplier sRNA:

- Target for use with Roche-NimbleGen expression arrays or other microarray platforms.
- RT-PCR studies.
- Sequencing template.
- Construction of a cDNA library.
- Archive valuable RNA samples for future use.

dsDNA Conversion Kit for RiboMultiplier sRNA Process

An overview of the RiboMultiplier Sense-RNA Amplification Kit process is shown in Fig. 1.

1. First-strand cDNA is synthesized from 5-20 µg of sRNA that has been produced using the RiboMultiplier Sense-RNA Amplification Kit. The reverse transcription reaction uses a primer (1st-Strand cDNA Primer) that anneals to the 3' poly(A) tail of the sRNA.
2. The cDNA produced is converted to dsDNA. The reaction uses a primer (2nd-Strand dsDNA Primer) that anneals to the Terminal Tagging Sequence present at the 5' end of the sRNA that has been produced by the RiboMultiplier Sense-RNA Amplification Kit.
3. The dsDNA is purified, e.g., by using spin columns (supplied by the user).

Preparation

Input RNA and dsDNA Yield:

The dsDNA Conversion Kit for RiboMultiplier sRNA efficiently converts the amplified sRNA produced by the RiboMultiplier Sense-RNA Amplification Kit to full-length dsDNA.

Table 1. Double-strand DNA yields obtained using the dsDNA Conversion Kit for RiboMultiplier™ sRNA. Results are based on multiple experiments using sRNA produced using the RiboMultiplier Sense-RNA Amplification Kit.

Input RiboMultiplier RNA	Average Yield of dsDNA
5 µg	2.4 µg
10 µg	3.0 µg
20 µg	3.2 µg

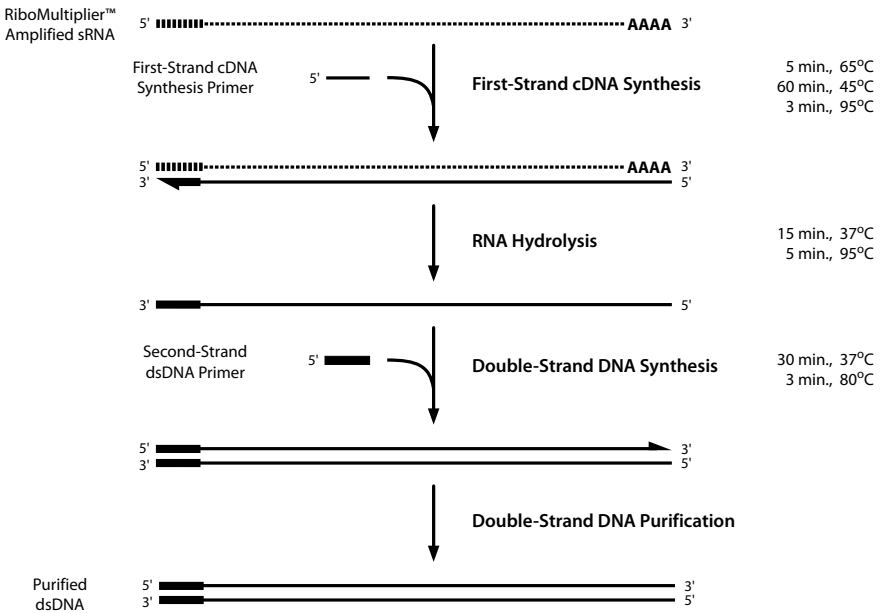


Figure 1. The dsDNA Conversion for RiboMultiplier™ sRNA Procedure.

Additional Suggestions:

Familiarize Yourself with the dsDNA Conversion Kit For Ribomultiplier sRNA Procedure:

The dsDNA Conversion Kit for RiboMultiplier sRNA includes many reagents. Before starting, please read this protocol carefully and familiarize yourself with each kit component and in which step of the process it is used. Be sure to wear gloves when handling the kit components.

Performing the dsDNA Conversion Kit for RiboMultiplier sRNA Kit Reaction:

We recommend that all reactions be performed in sterile 0.2 ml thin-walled tubes using sterile pipette tips and recently calibrated pipettors. Very small volumes of some kit components are required for each reaction. Therefore, we recommend you prepare Master Mixes of reaction components when working with multiple samples.

dsDNA Conversion Kit For Ribomultiplier sRNA Procedure**Performing a dsDNA Conversion Kit Reaction Using a Thermocycler**

The entire dsDNA Conversion Kit for RiboMultiplier sRNA reaction can be performed using a thermocycler. When using a thermocycler, we recommend the following cycling profile:

65°C for 5 minutes 45°C hold/pause (for reagent addition) 45°C for 60 minutes 95°C for 3 minutes 37°C hold/pause (for reagent addition) 37°C for 15 minutes 95°C for 5 minutes 37°C hold/pause (for reagent addition)	Part A First-Strand cDNA Synthesis
37°C for 30 minutes 80°C for 3 minutes	Part B Double-Strand DNA Synthesis

A. First-Strand cDNA Synthesis

The sRNA sample must be free of contaminating salts, metal ions, ethanol, and phenol. For best results, the RNA sample should be dissolved in RNase-Free Water.

Required in Part A

Component Name	Tube Label	Tube Color
1 st -Strand cDNA Primer	1 st -Strand cDNA Primer	Red
StarScript Reverse Transcription Buffer	StarScript RT Buffer	Red
dNTP PreMix	dNTP PreMix	Red
StarScript Reverse Transcriptase	StarScript RT	Red
RiboGuard RNase Inhibitor	RiboGuard RNase Inhibitor	Red
cDNA Finishing Solution	cDNA Finishing Solution	Red
RNase-Free Water	RNase-Free Water	Colorless

Thermocycler settings: 65°C for 5 minutes, 45°C for 60 minutes, 95°C for 3 minutes, 30°C for 15 minutes, and 95°C for 5 minutes.

- Anneal 1st-Strand cDNA Primer to the RiboMultiplier sRNA sample.
 - x µl RNase-Free Water
 - x µl RiboMultiplier sRNA sample (5-20 µg)
 - 0.5 µl 1st-Strand cDNA Primer

 12 µl Total volume

- Incubate at 65°C for 5 minutes, then cool the samples to 45°C. Maintain them at 45°C until the 1st-Strand cDNA Synthesis Master Mix is added. During the incubation, prepare the 1st-Strand cDNA Synthesis Master Mix as described in Part A, Step 3.
- Prepare the 1st-Strand cDNA Synthesis Master Mix.
For each 1st-strand cDNA synthesis reaction, combine on ice:

0.5	µl	RNase-Free Water
4	µl	StarScript Reverse Transcription Buffer
2	µl	dNTP PreMix
1	µl	RiboGuard RNase Inhibitor
0.5	µl	StarScript Reverse Transcriptase
8	µl	Total volume
- Gently mix the 1st-Strand cDNA Synthesis Master Mix and then add 8 µl of it to each reaction while the reaction is paused at 45°C.
- Gently but thoroughly mix each reaction and incubate at 45°C for 60 minutes.
- Incubate each reaction at 95°C for 3 minutes, then cool the samples to 37°C. Maintain them at 37°C until the cDNA Finishing Solution is added.
- Add 0.5 µl of cDNA Finishing Solution to each reaction while the reaction is paused at 37°C. Gently but thoroughly mix each reaction and incubate at 37°C for 15 minutes.
- Incubate each reaction at 95°C for 5 minutes, then cool the samples to 37°C. Maintain them at 37°C until the Double-Strand DNA Synthesis Master Mix is added. During the incubation, prepare the Double-Strand DNA Synthesis Master Mix as described in Part B, Step 1.

B. Double-Strand DNA Synthesis

Required in Part B

Component Name	Tube Label	Tube Color
2 nd -Strand dsDNA Primer	2 nd -Strand dsDNA Primer	Blue
DNA Polymerase	DNA Polymerase	Blue
Dithiothreitol	DTT	Blue

Thermocycler settings: 37°C for 30 minutes and 80°C for 3 minutes.

- Prepare the Double-Strand DNA Synthesis Master Mix.
For each reaction, combine on ice:

1	µl	2 nd -Strand dsDNA Primer
2.5	µl	DTT
1	µl	DNA Polymerase
4.5	µl	Total volume
- Gently mix the Double-Strand DNA Synthesis Master Mix and then add 4.5 µl of it to each reaction while the reaction is paused at 37°C.
- Gently but thoroughly mix each reaction and incubate at 37°C for 30 minutes.
- Incubate each reaction at 80°C for 3 minutes. Proceed to purification of the dsDNA.

C. Double-Strand DNA Purification

The dsDNA can be purified using, for example, the QIAquick PCR Purification Kit (Qiagen). If using this kit, follow the manufacturer's procedure with the following exception: the elution is performed using two aliquots of 30 µl each of EB buffer.

Related Products

RiboMultiplier™ Sense-RNA Amplification Kit

RM80510

10 Reactions

The kit will produce microgram amounts of sense-RNA (sRNA) from as little as 10 ng of total input RNA.

RiboMultiplier™ dsDNA Production System

RMS80601

1 System (2 Kits)

This system is sufficient for ten RiboMultiplier sRNA amplification reactions and then converting up to 500 µg of the amplified sRNA to full-length dsDNA.

MasterPure™ RNA Purification Kit

MCR85102

100 Purifications

This kit provides all reagents needed to purify total RNA from >10,000 eukaryotic cells without the use of columns or organic solvents such as phenol.

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726 Post Road, Madison, WI 53713 (800) 284-8474 (608) 258-3080 Fax (608) 258-3088