RScript™ II Reverse Transcriptase 50,000U/10,000U

01 APR 2024

Catalog Number	Size	Concentration
RT002-0250	250 rxns	200 U/μl
RT002-0050	50 rxns	200 U/μl

Storage Conditions

Stable for up to 2 years at -20°C

Description

Overcome the most challenging RNA structures over a wide temperature range.

Bio-Helix RScript II Reverse Transcriptase - Engineered innovatively and specifically for both Research and Diagnostic applications for meeting all your cDNA synthesis needs and for overcoming the most challenging secondary RNA structures over a wide temperature range. Our next-generation, engineered recombinant reverse transcriptase, with improved thermostability, processivity, robustness, optimal cDNA yields, proprietary site mutations for reduced RNase H activity, and extended half-life, is the most versatile reverse transcriptase in the world for not only simply meeting the routine cDNA synthesis requirements but also enabling superior performance for even the most challenging RNA samples at hand.

Kit Content(s)

RT002-0250	RScript II Reverse Transcriptase	50 μl x 5 vials
	5X Sharp II reaction buffer	250 μl x 5 vials
RT002-0050	RScript II Reverse Transcriptase	50 μl x 1 vial
	5X Sharp II reaction buffer	250 μl x 1 vial

Required materials but not provided

- Vortex or equivalent
- Microcentrifuge
- PCR tubes for your instruments
- Ice water bath
- Temperature-controlled water bath or heat blocks; the thermal cycler can also be used.

Template

Total RNA, synthetic RNA transcript or poly(A)+mRNA, or the RNA should be avoided for cross-contamination with DNA.

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Primer Selection

Primer amounts recommended for efficient cDNA synthesis are 0.5ug of oligo(dT) (anneal to the 3'-poly(A) + mRNA) or random primers (anneal at non-specific sites of RNA templates), or 2 uM of gene-specific primers per 20 ul reaction.

Reaction Setup

Simple setup for cDNA Synthesis

1. For each 20 ul cDNA synthesis reaction, assemble the following in a DNase/RNase-free tube. Keep it on ice just prior to use.

Component	Volume	Final conc.
RNA template	X µl	≦ 1 µg total RNA or ≦ 0.1µg poly(A) mRNA
5X Sharp II reaction buffer	4 µl	
Primers*	1 µl	
RScript II Reverse Transcriptase	1 µl	200 U
RNase Inhibitor	0.5 µl	20 U
dNTPs Mix (10 mM)	1 µl	0.2 mM
Nuclease-Free Water	Add to 20 µl	
Total volume	20 µl	

* Depending on the purpose of the experiment, 1 µl of Oligo18 (dT) or 1 µl of Random Primer can be added as the primer or 1 µl of the mixed primer may be added after mixing Oligo18 (dT) and Random Primer with a certain ratio. Alternatively, 1 µl of the self-prepared sequence-specific primers (20 µM) can be added as needed for the experiment. When using the self-prepared sequence-specific primers, the RNA sample amount can be adjusted to "≤ 5 µg total RNA or ≤ 0.5 µg poly(A) mRNA".

2. Mix the reaction solution gently by pipetting.
3. Incubate at 50°C for 15 minutes if using Oligo18 (dT) or sequence-specific primers.
   Incubate at 25°C for 10 minutes or at 50°C for 15 minutes if using the Random Primer
   Note: It is recommended to increase the complex template' s reverse transcription temperature to 55-60°C. The reaction time can be adjusted according to the experimental applications.
4. The reaction tube from the Step 3 must be incubated at 85°C for 5 minutes for inactivating the Reverse Transcriptase before amplification. At the end of the reaction, the resulting cDNA should be placed on ice for subsequent experiments or cryopreservation.

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Standard setup for cDNA Synthesis:

By following this procedure, it' ll help you unwind the secondary structure of complex RNA templates, improve reverse transcription efficiency, and increase the length of cDNA products.

1. For cDNA synthesis reaction, assemble the following in a DNase/RNase-free tube. Keep it on ice just prior to use.

Component	Volume	Final conc.
RNA template	X µl	≦ 1 µg total RNA or ≦ 0.1µg poly(A) mRNA
Primers*	1 µl	
dNTPs Mix (10 mM)	1 µl	0.2 mM
Nuclease-Free Water	Add to 10 µl	
Total volume	10 µl	

*Depending on the purpose of the experiment, 1 µl of Oligo18 (dT) or 1 µl of Random Primer can be added as the primer or 1 µl of the mixed primer may be added after mixing Oligo18 (dT) and Random Primer with a certain ratio. Alternatively, 1 µl of the self-prepared sequence-specific primers (20 µM) can be added as needed for the experiment. When using the self-prepared sequence-specific primers, the RNA sample amount can be adjusted to "≤ 5 µg total RNA or ≤ 0.5 µg poly(A) mRNA".

2. Incubated at 65°C for 5 minutes then incubated in an ice bath for 2 minutes.
3. Add the materials below in the reaction PCR tube

Component	Volume	Final conc.
Reaction liquid from step 2	10 µl	
5X Sharp II reaction buffer	4 µl	
RNase Inhibitor	0.5 µl	20 U
RScript II Reverse Transcriptase	1 µl	200 U
Nuclease-Free Water	Add to 20 µl	
Total volume	20 µl	

4. Mix the reaction solution gently by pipetting.
5. Incubate at 50°C for 30 -50 minutes if using Oligo18 (dT) or sequence-specific primers.
   Incubate at 25°C for 10 minutes or at 50°C for 30 - 50 minutes if using the Random Primer.
   Note: The reaction time can be adjusted appropriately according to the experimental applications. If the synthesized cDNA is used as a qPCR template, the reaction conditions are then incubated at 50°C for 15 min, refer to the Simplified Procedures.
6. The reaction tube from Step 5 must be incubated at 85°C for 5 minutes to inactivate the Reverse Transcriptase before amplification. At the end of the reaction, the resulting cDNA should be placed on ice for subsequent experiments or cryopreservation.