



RScript™ cDNA Synthesis Kit (Premix)

Catalog Number	Size
RK002-0100	100 rxns

Storage Conditions

Stable for up to 2 years at -20°C

Description

This product is a premixed reagent for the first-strand cDNA synthesis developed based on the RScript II Reverse Transcriptase. RScript II Reverse Transcriptase has a higher thermal stability than the RScript Reverse Transcriptase. It can optimize the reverse transcription temperature to 55-60°C for complex secondary structures and high GC content targets, overcome the inhibition of cDNA synthesis by complex RNA secondary structures, and effectively synthesize the high-quality cDNA. RScript II Reverse Transcriptase has a higher cDNA synthesis capacity, making it ideal for the reverse transcription of small amounts of template as well as low-copy genes. This product is provided conveniently in the form of premixed reagents - 2× Sharp Buffer (with the primers) containing an optimized buffer system, dNTPs, and premix of the random primer and Oligo18 (dT). RScript II RT Mix contains the ratio-optimized RScript II Reverse Transcriptase and RNase inhibitor, enabling the synthesis of the first-stranded cDNA from very low amounts of total RNA or poly(A) mRNA. The synthesized single-stranded cDNA product can be used directly for subsequent PCR or qPCR reactions.

Kit Content(s)

RK002-0100	RScript II Enzyme Mix	100 µl x 1 vial
	2X Sharp Reaction Mix	1 ml x 1 vial
	Nuclease-free water	1.5 ml 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 RNA should be avoided for cross-contamination with DNA.



Reaction Setup

cDNA Synthesis

1. For each 20 μ l cDNA synthesis reaction, assemble the following in an RNase free tube. Keep it on ice just prior to use.

Component	Volume	Final conc.
RNA template	X μ l	$\leq 1\mu\text{g}$ total RNA or $\leq 0.1 \mu\text{g}$ poly(A) mRNA
2X Sharp Reaction Mix	10 μ l	
RScript II Enzyme Mix	1 μ l	
Nuclease-Free Water	Add to 20 μ l	
Total volume	20 μ l	

2. Mix the reaction solution gently by pipetting.
3. Cap the tubes and centrifuge briefly. Place them in the temperature-controlled water bath or heat blocks. Incubate the tubes at 50°C for 15-50 mins for the extension step.

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. For example, if the synthetic cDNA is used as a qPCR template, the reaction condition is the incubation at 50°C for 15 minutes.

4. The reaction tube from Step 3 must be incubated at 85°C for 5 minutes to inactivate the RScript II Reverse Transcriptase before amplification.