01 APR 2024

RScript™ cDNA Synthesis Kit (Premix + gDNA Remover)

Catalog Number	Size
RK003-0100	100 rxns

Storage Conditions

Stable for up to 2 years at -20°C

Description

This product is a one-tube master mix for genomic elimination and reverse transcription, which can achieve genomic DNA removal and cDNA synthesis at the same time in a single-step operation, effectively avoiding the risk of sample contamination and RNA degradation caused by complex additions. The product contains a highly potent RScript II reverse transcriptase, which synthesizes the first-strand cDNA from very low amounts of total RNA or poly(A) mRNA, making it suitable for the reverse transcription of RNA templates with high-GC contents and complicated structures. The enzyme has greatly improved thermal stability, able to tolerate the reaction temperature up to 65°C and achieve a higher yield of synthesized cDNA. With the addition of the heat-sensitive ds DNase, the residual genomic DNA can be removed from the sample at room temperature. The inactivation at 50°C does not affect the cDNA. The optimized 5×Sharp Reaction Mix is premixed with the Random Primer and Oligo18 (dT), compatible with genomic digestion and cDNA synthesis. Reverse transcripts are compatible with dye and probe qPCR for subsequent gene expression analysis.

Kit Content(s)

RK003-0050	RScript II Enzyme Mix	100 µl x 1 vial
	5X Sharp Reaction Mix	400 µl x 1 vial
	No RT Control Mix	10 µl 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 the RNA should be avoided for cross-contamination with DNA.

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Reaction Setup

cDNA Synthesis

1. For each 20 ul 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 µl	≦ 1µg total RNA or ≦ 0.1 µg poly(A) mRNA
5X Sharp Reaction Mix	4 µl	
RScript II Enzyme Mix	1 µl	
Nuclease-Free Water	Add to 20 µl	
Total volume	20 µl	

2. No RT Control reaction

Component	Volume	Final conc.
RNA template	X µl	≦ 1µg total RNA or ≦ 0.1 µg poly(A) mRNA
5X Sharp Reaction Mix	4 µl	
No RT Control Mix	1 µl	
Nuclease-Free Water	Add to 20 µl	
Total volume	20 µl	

Note: This reaction is a negative control reaction without the reverse transcriptase and is used to check for genomic residues in the RNA template.

3. Mix the reaction solution gently by pipetting.
4. Cap the tubes and centrifuge briefly. Place them in the temperature-controlled water bath or heat blocks. Incubate the tubes as follows:

Temperature	Time
37°C	2 min
50°C *	15 min
85°C	2 min

* Complex templates can be performed at 55-60°C to improve the reverse transcription efficiency. The reverse transcription time of high-expression genes can be shortened to 5-10 minutes, which improves the experimental efficiency.

5. At the end of the reaction, the resulting cDNA should be placed on ice for the subsequent experiment(s) or cryopreservation.

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