RScript cDNA Synthesis Kit

20 DEC 2022

Catalog Number	Size	Concentration
RK001-0050	50 rxns	200 U/µl

Storage Conditions

Stable for up to 1.5 years at -20°C

Description

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

Bio-Helix RScript cDNA Synthesis Kit - 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 – is the latest addition to our reverse transcription product boutique. The RScript cDNA Synthesis Kit contains our next-generation, engineered recombinant M-MLV 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)

RK001-0050	RScript Enzyme Mix	100 µl x 1 vial
	2X Sharp Reaction Mix	500 µl x 1 vial
	Oligo $d(T)_{20}$ Primer (50 uM)	50 µl x 1 vial
	Random Hexamer Primer (50 ng/ul)	50 µl x 1 vial
	Nuclease-free water	1 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.

Primer Selection

Primer amounts recommended for efficient cDNA synthesis are 2.5 uM of oligo(dT) (anneal to the 3'-poly(A) + mRNA) or 2.5 ng/ul of random primers (anneal at non-specific sites of RNA templates), or 2 uM of gene-specific primers per 20 ul reaction.

Reaction Setup

cDNA Synthesis

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

Component	Final conc.	Volume
RNA template*	10 pg-2 ug total RNA or 10 pg-500 ng mRNA	X ul
2X Sharp Reaction Mix (including dNTPs, $MgCl_2$)*		10 ul
Primers		1 ul
RScript Enzyme Mix	200 U	2 ul
RNase Inhibitor	20-40 U	1 ul
Nuclease-Free Water		Add to 20 ul
Total volume		20 ul

*RNA template, primer and 2X Sharp Reaction Mix need to be premixed and heated at 65°C for 5 minutes in advance. After RNA has been pre-heated and incubated on ice bath at least 1 minute, add other components according to the table.

2. Mix the reaction solution gently by pipetting.
3. Cap the tubes and place them in the temperature-controlled water bath or heat blocks. Incubate the tubes at 55°C for 50 mins for the extension step. The 42°C – 60°C temperature range may be the optimal temperature for extension.
4. The reaction tube from the Step 3 must be incubated at 70°C for 15 minutes for inactivating the Reverse Transcriptase before amplification.

Storage Buffer

The enzyme is supplied in a storage buffer consisting of 20 mM Tris-HCl (pH 7.4), 0.1 M NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) NP-40 and 50% (v/v) glycerol.