MQ cDNA Synthesis Kit

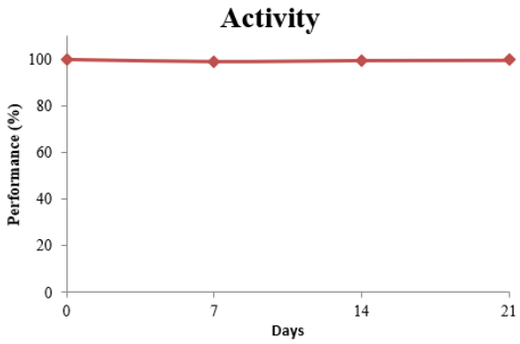
Cat. No. CDS-M-003-100
Size: 100 Preparations
Store at -20°C under dark condition

MOLEQULE-ON®

Kit Components	Quantity
MQ RScript Enzyme Mix	100ul x 2
5X Sharp Reaction Mix	200ul x 2
Oligos $d(T)_{20}$ Primer (50 uM)	50ul x 2
Random Hexamer Primer (50ng/ul)	50ul x 2
Nuclease free Water	1ml x 2

Description

MQ cDNA Synthesis Kit is engineered innovatively and specifically for Research and Laboratory applications for meeting all your cDNA synthesis needs and for overcoming the most challenging secondary RNA structures over a wide temperature range. The 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.



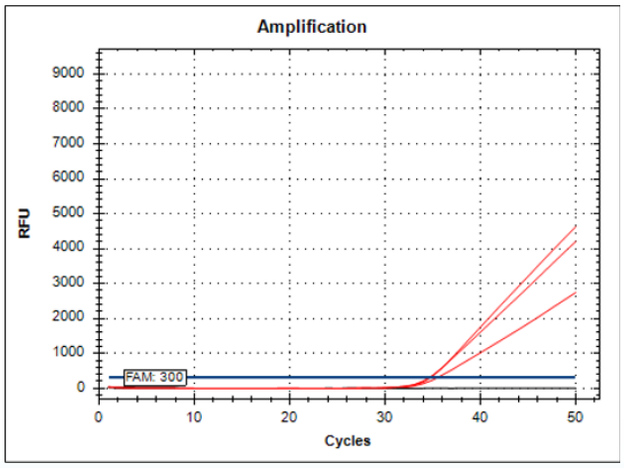
RScript@37°C	Day7		Day14		Day21	
	10^ -2	10^ -5	10^ -2	10^ -5	10^ -2	10^ -5
RT-qPCR Ct	22.528	31.395	21.860	31.224	22.725	31.701
	22.411	31.077	21.926	30.904	22.586	31.806
Activity	99.10%		99.53%		99.63%	

Stability of MQ RScript Reverse Transcriptase

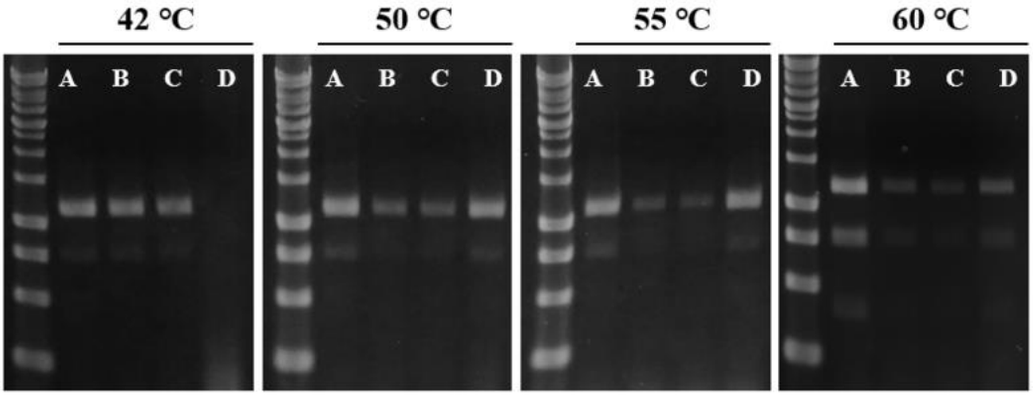
The stability RT-qPCR data shows the performance of MQ RScript reverse transcriptase is maintained without any significant alteration in performance at 37 °C after a period of 3 weeks (21 days), demonstrating its high resistance to temperature and time. The percentage of MQ RScript reverse transcriptase activity was calculated by dividing values at each reaction temperature.

Performance of One-Step RT-qPCR with MQ RScript Reverse Transcriptase

To evaluate the RT performance for the COVID-19 fragments, the N1 gene with 100 copies per reaction was tested in the one-step RT-qPCR. Experiments proved to be feasible and effective (mean Ct : 34.88).



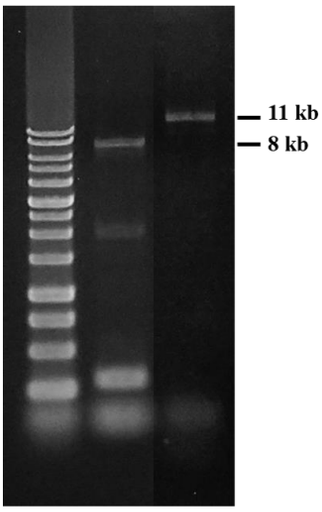
For Laboratory Use Only



A: MQ Rscript Reverse Transcriptase Cat# EN-M-005-5000
B: Competitor 1
C: Competitor 2
D: Competitor 3

Enhancement of the Thermostability

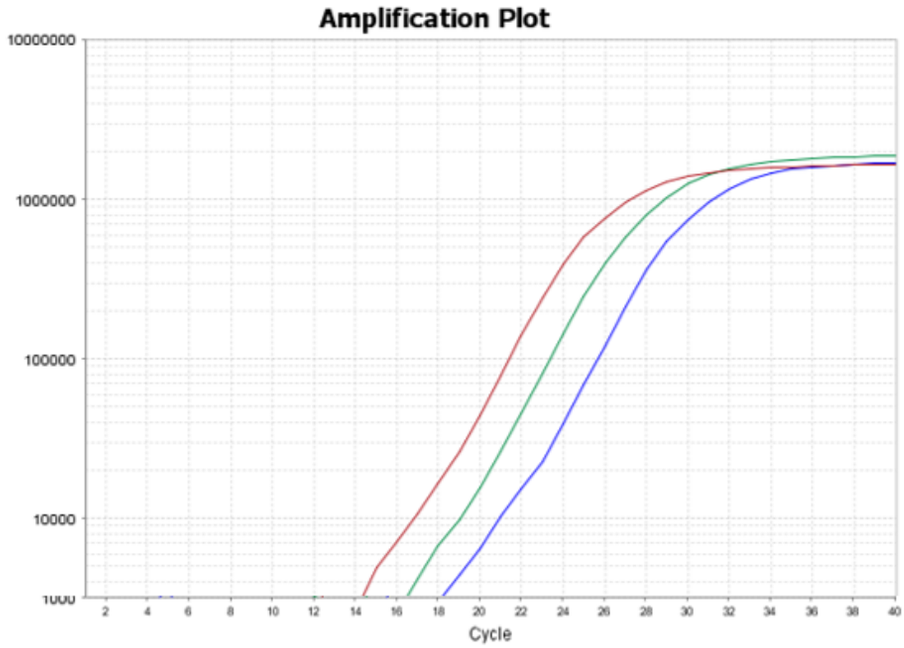
Reverse transcription of a 1 kb fragment ARHGAP29 RNA using MQ RScript reverse transcriptase or competitor reverse transcriptases were used to carry this experiment. Regardless of the variations in temperatures (42 -60 °C) MQ RScript reverse transcriptase shows a stable and superior performance at higher temperatures of up to 60 ℃ compared to other brands. The molecular weight marker used was DNA Ladder 1kb.



Elongation of RNA templates up to 11 kb in length

MQ RScript reverse transcriptase was used in a reaction with a range of human bladder cancer cell lines - 5637 (HTB-9) RNA. The resulting synthesized cDNA (8 kb ,11kb) was followed by PCR and visualized on a 2% agarose gel. The molecular weight marker used was DNA Ladder 1kb.

At a temperature of 55 °C, MQ RScript Reverse Transcriptase exceeds performance demonstrating better efficiency and higher cDNA yields. MQ RScript Reverse Transcriptase shows a stable and superior performance at 55 ℃ compared to other brands. Therefore MQ RScript reverse transcriptase does not encounter significant higher Ct values compared to other brands due to their low amounts of input cDNA.



Performing RT-qPCR with MQ RScript Reverse Transcriptase

Red: MQ RScript Reverse Transcriptase #EN-M-005-5000
Green: Competitor 1
Blue: Competitor 2

Required materials but not provided

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

Procedure

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

Components	20 µl reaction	Final Conc.
RNA Template	X µl	10 pg – 2 µg total RNA or 10 pg – 500 ng mRNA
5X Sharp Reaction Mix	4 µl	1X
Primers	1 µl	
MQ RScript Enzyme Mix	2 µl	200 U
RNase Inhibitor	1 µl	20 - 40 U
Nuclease Free Water	Add to 20 µl	
Total Volume	20 µl	

*RNA template, primer and 5X 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.