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AccuPower® *RocketScript*™ Cycle RT PreMix

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I. Introduction

AccuPower® *RocketScript*™ Cycle RT PreMix is a ready-to-use lyophilized mastermix containing all components for first-strand cDNA synthesis from purified Poly(A) or total RNA template. Oligo $(dT)_{20}$ is also included in the premix for added convenience. Simply add your template and DEPC-water to begin your reaction.

The *AccuPower RocketScript* Cycle RT PreMix contains *RocketScript* Reverse Transcriptase, a new *M-MLV* originated Reverse Transcriptase that has been engineered to provide increased thermal stability in order to synthesize full length first-strand cDNA more efficiently. The amount of starting material can vary from 1 pg to > 1 µg of total RNA and RNA targets from 100 bp to > 10 kb can be detected with the *AccuPower RocketScript* Cycle RT PreMix.

The *AccuPower RocketScript* RT PreMix can be used to synthesize cDNA at a temperature range of 42 – 70°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases.

II. Application

- Standard RT and RT-PCR
- Real-Time PCR
- Synthesis of double-stranded cDNA for cloning
- Gene expression level analysis

III. Contents

Component	Amount
RocketScript Reverse Transcriptase	200 U
Oligo $(dT)_{20}$	100 pmole
5 x Reaction Buffer	1 x
DTT	0.25 mM
dNTP	250 µM each
RNase Inhibitor	1 U

IV. Principle

RocketScript Reverse Transcriptase in the *AccuPower RocketScript* RT PreMix is genetically engineered thermal stable *M-MLV* Reverse Transcriptase with enhanced thermal stability and outstanding processivity. The enzyme also features increased specificity and improved efficiency allowing efficient reverse transcription of RNA molecules with complex secondary structures.

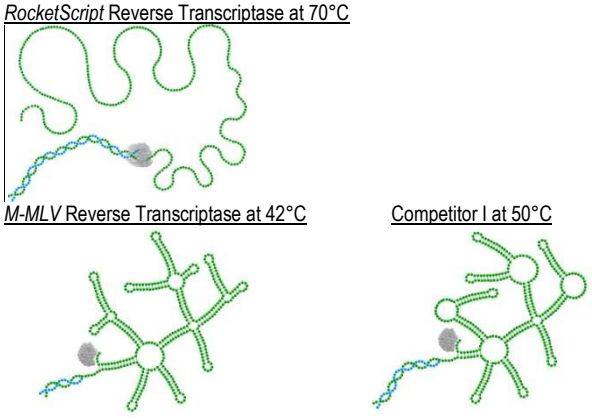

Figure 1. Schematic representation of the 5'UTR of a gene, with complex secondary structure, at three different temperatures. Note that *RocketScript* Reverse Transcriptase shows full activity at 70°C allowing it to synthesize the complete gene sequence where *M-MLV* and other Reverse Transcriptases fail.

V. Storage

AccuPower RocketScript Cycle RT PreMix should be stored at -20°C upon receipt and is stable until the expiry date stated on the label.

VI. Notice to Purchaser

AccuPower RocketScript Cycle RT PreMix exhibits RNase H activity and contains oligo $(dT)_{20}$ primer.

VII. Additional Required Materials & Devices

- Thermal cycler for PCR
- Calibrated micropipette
- Sterilized micropipette tips with filters

VIII. General Precautions

- Wear gloves during experiments to prevent contamination.
- Store positive materials, such as samples and control templates, in separated freezer from freezers for the kit.
- Add templates to the reaction mixture in clean bench or a spatially separated facility.

IX. Protocol

1. Thaw total RNA and DEPC-water before use.
2. Add 10 pg – 5 µg of total RNA into the *AccuPower RocketScript* Cycle RT PreMix tubes.
3. Add DEPC-water into the *AccuPower RocketScript* Cycle RT PreMix tubes to a total volume of 20 µl (K-2201, K-2202) or 50 µl (K-2203, K-2204). Do not calculate the dried pellet.
4. Dissolve the lyophilized pellet completely and spin down by using Bioneer's *ExiSpin* Vortex/Centrifuge or by pipetting up and down several times and briefly spinning down.
5. Perform the reaction under the following conditions.

Cyclic Temperature Reverse Transcription (CTRT) method option A

Step	Temperature	Time	No. of Cycles
Primer annealing	37°C	10 – 30 sec	> 10
cDNA synthesis	50°C	4 min	
Denaturation of RNA secondary structure and cDNA synthesis	55 – 60°C	30 sec	
Heat inactivation	95°C	5 min	1

Cyclic Temperature Reverse Transcription (CTRT) method option B

Step	Temperature	Time	No. of Cycles
Primer annealing	37°C	1 min	> 10
cDNA synthesis	42 – 70°C	4 min	
Heat inactivation	95°C	5 min	1

6. Maintain the reaction at 4°C after amplification. The sample can be stored at -20°C until use.

X. Reaction Example

1. Reaction mixture

Component	Volume	Concentration
Template RNA	1 µl	100 pg/µl
DEPC-D.W	19 µl	
Total	20 µl	

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2. Reaction condition

Step	Temperature	Time	No. of Cycles
Primer annealing	37°C	10 sec	> 10
cDNA synthesis	50°C	4 min	
Denaturation of RNA secondary structure and cDNA synthesis	60°C	30 sec	
Heat inactivation	95°C	5 min	1

XI. Experimental Data

Template RNA	*RocketScript* Cycle RT	Supplier Q	Supplier I
10 ng	23.91	25.63	24.43
1 ng	27.33	28.92	28.03
100 pg	30.62	32.42	30.88
10 pg	33.63	35.43	33.95
Efficiency	104%	103%	108%
Linearity	0.99999	0.9996	0.9995

Figure 2. Sensitivity comparison between *AccuPower RocketScript* Cycle RT PreMix and other suppliers' products.
Reverse transcription reactions were performed as follows: Incubation at 60°C for 1 hr, inactivation at 95°C for 5 min. All cDNAs were amplified with *AccuPower DualStar*™ qPCR PreMix (K-6110) from Bioneer.

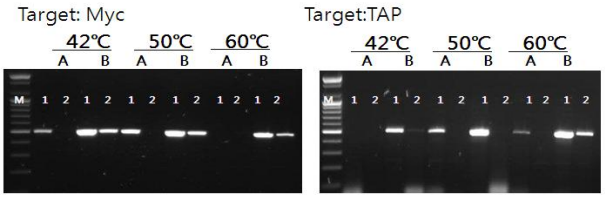
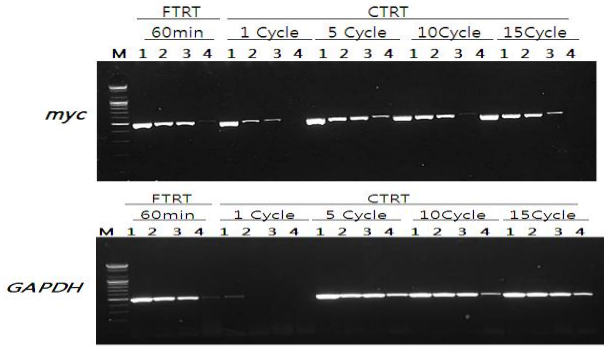


Figure 3. Complex RNA amplification using *AccuPower RocketScript* Cycle RT PreMix. RNA was isolated from each target gene and then used to synthesize cDNA using *AccuPower RocketScript* Cycle RT PreMix and *M-MLV* Reverse Transcriptase for gene expression analysis. Reverse transcription reactions were performed as follows: Incubation at each temperature 42, 50, 60°C for 1 hr, inactivation at 95°C for 5 min. All cDNAs were amplified with *AccuPower* Hotstart PCR PreMix (K-5050) from Bioneer.

A: *M-MLV* Reverse Transcriptase
B: *AccuPower RocketScript* Cycle RT PreMix with Oligo $(dT)_{20}$
Primer set: Human Myc and TAP / Human Total RNA from HeLa cell
Lane M: 100 bp DNA Ladder
Lane 1: 100 ng
Lane 2: 10 ng



FTRT(Fixed Temperature Reverse Transcription) reaction

Step	Temperature	Time	No. of Cycles
cDNA synthesis	50°C	60 min	1
Heat inactivation	95°C	5 min	1

CTRT(Cyclic Temperature Reverse Transcription) reaction

Step	Temperature	Time	No. of Cycles
Primer annealing	37°C	10~30 sec	1, 5, 10, or 15
cDNA synthesis	50°C	4 min	
Denaturation of RNA secondary structure and cDNA synthesis	55°C	30 sec	
Heat inactivation	95°C	5 min	1

Primer set : Human GAPDH, myc set
Human Total RNA from HeLa cell
Lane M : 1 kb DNA Ladder
Lane1: 10 ng / Lane 2: 1 ng / Lane 3: 100 pg / Lane 4: 10 pg

Figure 4. Sensitivity comparison of cDNA synthesis using FTRT and CTRT methods.
RNA was isolated from each target gene and then used to synthesize cDNA using *AccuPower RocketScript* Cycle RT PreMix. cDNA synthesis was performed from serially diluted RNA template using FTRT and CTRT methods. Note that CTRT with 5, 10, and 15 cycles synthesized higher yield cDNA than FTRT in both myc and GAPDH tests.

Figure 5. Comparison of amplification efficiency between *AccuPower RocketScript* Cycle RT PreMix and other suppliers' products.
All cDNAs were amplified with *AccuPower* Hotstart PCR PreMix (K-5050) from Bioneer.

Primer set : Human Myc 498 bp set
Human Total RNA in HeLa Cell
Lane M : 1 kb ladder
Lane 1: 10 ng Lane 2: 1 ng Lane 3: 100 pg Lane 4: 10pg

XII. Ordering Information

Cat. No.	Description
K-2201	*AccuPower RocketScript* Cycle RT PreMix, 20 µl, 12 x 0.2 ml thin-wall 8-strip tubes with attached cap (96 rxns)
K-2202	*AccuPower RocketScript* Cycle RT PreMix, 20 µl, 60 x 0.2 ml thin-wall 8-strip tubes with attached cap (480 rxns)
K-2203	*AccuPower RocketScript* Cycle RT PreMix, 50 µl, 12 x 0.2 ml thin-wall 8-strip tubes with attached cap (96 rxns)
K-2204	*AccuPower RocketScript* Cycle RT PreMix, 50 µl, 60 x 0.2 ml thin-wall 8-strip tubes with attached cap (480 rxns)

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