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I. Introduction

AccuPower® RocketScript™ 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. Simply add your template, primer, and DEPC-water to begin your reaction.

The AccuPower RocketScript 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 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

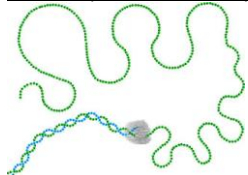
III. Contents

Component	Amount
RocketScript Reverse Transcriptase	200 U
5 x Reaction Buffer	1 x
DTT	0.25 mM
dNTP	250 µM each
RNase Inhibitor	1 U

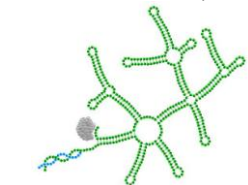
IV. Principle

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.

RocketScript Reverse Transcriptase at 70°C



M-MLV Reverse Transcriptase at 42°C



Competitor I at 50°C

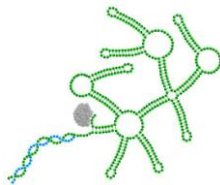


Figure 1. Schematic representation of the 5'UTR of a gene, with complex secondary structure, at three different temperatures. Note that RocketScript shows full activity at 70°C allowing it to synthesize the complete gene sequence where M-MLV and other Reverse Transcriptase's fail.

V. Storage

AccuPower RocketScript 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 RT PreMix exhibits RNase H activity.

VII. Additional Required Materials & Devices

- Thermal cycler for PCR
- Target-specific primers, oligo dT, random hexamer, or nonamers
- 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, DEPC-water, and primer before use.
2. Add total RNA and primer (oligo dT, random primer, or specific primer) into the AccuPower RocketScript RT PreMix tubes.

Recommended amount of template and primer

Components	Amount
Total RNA	10 pg – 5 µg
Oligo dT or random primer	10 – 100 pmoles
Specific primer	10 – 50 pmoles

3. Add DEPC-water into the AccuPower RocketScript RT PreMix tubes to a total volume of 20 µl (K-2101, K-2102) or 50 µl (K-2103, K-2104). 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.

Step	Temperature				Time
	dN ₆	dN ₁₂	dT ₂₀	Specific primer	
Primer annealing	15°C	30°C	37°C	T _m of primer	1 min
cDNA synthesis	42 – 70°C				10 – 60 min
Heat inactivation	95°C				5 min

Alternative protocol

Step	Temperature	Time
cDNA synthesis	50°C	1 hr
Heat inactivation	95°C	5 min

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

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X. Reaction Examples

1. Reaction mixture

Component	Volume	Amount
Template RNA	1 µl	10 pg
dN ₁₂	1 µl	20 pmoles
DEPC-D.W	18 µl	
Total	20 µl	

2. Reaction condition

Step	Temperature	Time
Primer annealing	30°C	5 min
cDNA synthesis	60°C	1 hr
Heat inactivation	95°C	5 min

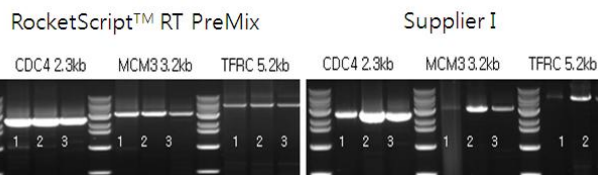


Figure 3. Comparison of long kb amplification between AccuPower RocketScript RT PreMix and Company I reverse transcriptase.

Reverse transcription reactions were performed according to each manufacturer's recommendation. All cDNAs were amplified with AccuPower HotStart PCR PreMix (K-5050) from Bioneer. Note supplier I shows inhibition with high input concentration of total RNA.

Lane 1: 1 µg Human total RNA from HeLa cell
Lane 2: 100 ng Human total RNA from HeLa cell
Lane 3: 10 ng Human total RNA from HeLa cell

XI. Experimental Data

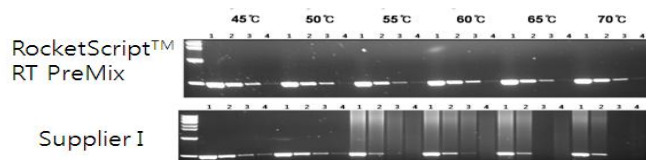


Figure 2. Amplification results of AccuPower RocketScript RT PreMix using myc compared with Company I reverse transcriptase.

Reverse transcription condition: Incubation at each temperature 45, 50, 55, 60, 65, 70°C for 1 hr, inactivation at 95°C for 5 min / Primer set: human myc 495 bp set

Lane M : 1 kb DNA Ladder
Lane 1: 100 ng Human total RNA from HeLa cell
Lane 2: 10 ng Human total RNA from HeLa cell
Lane 3: 1 ng Human total RNA from HeLa cell
Lane 4: 100 pg Human total RNA from HeLa cell

XII. Ordering Information

Cat. No.	Description
K-2101	AccuPower RocketScript RT PreMix, 20 µl, 12 x 0.2 ml thin-wall 8-strip tubes with attached cap (96 rxns)
K-2102	AccuPower RocketScript RT PreMix, 20 µl, 60 x 0.2 ml thin-wall 8-strip tubes with attached cap (480 rxns)
K-2103	AccuPower RocketScript RT PreMix, 50 µl, 12 x 0.2 ml thin-wall 8-strip tubes with attached cap (96 rxns)
K-2104	AccuPower RocketScript RT PreMix, 50 µl, 60 x 0.2 ml thin-wall 8-strip tubes with attached cap (480 rxns)

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