

AccuPower® RocketScript™ RT-PCR PreMix

Bioneer Corporation	Seoul Office	Bioneer, Inc.	Order
49-3, Munpyeong-dong, Daedeok-gu, Daejeon 306-220 Korea Phone:+82-42-936-8500 Fax: +82-72-930-8600	2nd fl. Samhwa&Sansu B/D, #6 Yangjae-dong, Seocho-ku Seoul, 137-130, Korea Phone:+82-2-598-1094 Fax: +82-2-598-1096	1000 Atlantic Avenue Alameda, CA 94501 USA Toll free : 1-877-264-4300 Fax : 1-510-865-0350 E-mail: order.usa@bioneer.us.com	Korea only: 1588-9788 E-mail: order@bioneer.co.kr URL: www.bioneer.com

I. Introduction

AccuPower® RocketScript™ RT-PCR PreMix is a ready-to-use lyophilized mastermix containing all components for first-strand cDNA synthesis and PCR reaction in one tube

AccuPower RocketScript RT-PCR PreMix is using RocketScript™ Reverse Transcriptase and Top DNA Polymerase. It is optimal to amplify the template RNA which has a secondary structure RNA and performs the best amplification regardless of the amount of template RNA.. It is suitable for expression of the low copy gene because RocketScript™ Reverse Transcriptase is genetically engineered, thermal stable M-MLV with enhanced thermal stability and can guarantee the precise and high efficiency of reverse transcription reaction from 10pg to 5 µg template RNA. This kit contains a special stabilization material and the activities of enzyme (RocketScript™ Reverse Transcriptase, Top DNA polymerase) last long enough. It has also very convenient and simple protocols so that it implements the best cDNA synthesis and PCR reaction.

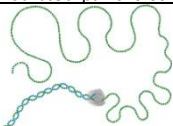
II. Application

- Standard RT-PCR
- Gene expression level analysis
- Single-Cell RT-PCR

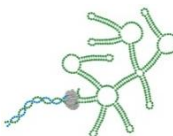
III. Principle

RocketScript Reverse Transcriptase in the AccuPower RocketScript RT-PCR 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 50°C



Competitor I at 50°C

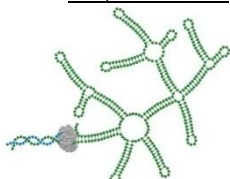


Figure 1. Schematic representation of the 5'UTR of a gene, with complex secondary structure, at three different temperatures.

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

IV. Storage

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

V. Additional Required Materials & Devices

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

VI. 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

VII. Protocol

[20 µl reaction volume]

1. Thaw Total RNA, DEPC-water and Specific primer before use.
2. Add Total RNA and Specific primer into AccuPower RocketScript RT-PCR PreMix tubes.

Components		Amount
Template RNA	Total RNA	10pg ~5µg
	Poly(a) RNA	10pg ~5µg
Specific Primer		10~30 pmoles

3. Add DEPC-water into AccuPower RocketScript RT-PCR PreMix tubes to a total volume of 20 µl. Do not calculate the dried pellet.
4. Dissolve the lyophilized White pellet by flick with your finger or pipetting, and briefly spin down.
5. Perform the reaction under the following conditions.

Step	Temperature	Time	Cycles
cDNA synthesis	42~70°C	10~60 min	1
Pre-Denaturation	95°C	5 min	1
Denaturation	95°C	10~30sec	30
Annealing	50~65°C	10~30sec	
Extension	72°C	1kb/1min	
Final extension	72°C	5min	1

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

Note: reaction temperature should be selected to fit the Tm value of Primers

VIII. Experimental data



Figure 2. Sensitivity comparison between AccuPower RocketScript RT-PCR PreMix and other suppliers' products

All reactions were carried out following the suppliers' instructions. / Primer set: human myc 495 bp set
Human Total RNA from Hela Cell
Lane 1: 10 ng Lane 2: 1 ng Lane 3: 100 pg Lane 4: 10 pg
Lane M : 1 kb DNA Ladder

IX. Ordering Information

Cat. No.	Description
K-2501	AccuPower® RocketScript™ RT-PCR PreMix, 0.2ml thin-wall 8-Strip tubes with attached cap, 20 µl, 96 tubes
K-2503	AccuPower® RocketScript™ RT-PCR PreMix, 0.2ml thin-wall 8-Strip tubes with attached cap, 50 µl, 96 tubes
K-2502	AccuPower® RocketScript™ RT-PCR PreMix, 0.2ml thin-wall 8-Strip tubes with attached cap, 20 µl, 480 tubes
K-2504	AccuPower® RocketScript™ RT-PCR PreMix, 0.2ml thin-wall 8-Strip tubes with attached cap, 50 µl, 480 tubes