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## I. Introduction

*AccuPower<sup>®</sup> 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<sup>™</sup>* 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<sup>™</sup>* 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<sup>™</sup>* 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.

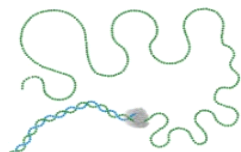
## II. Application

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

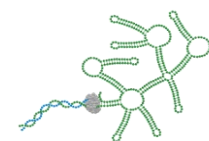
## III. Principle

*RocketScript* Reverse Transcriptase in the *AccuPower<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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.

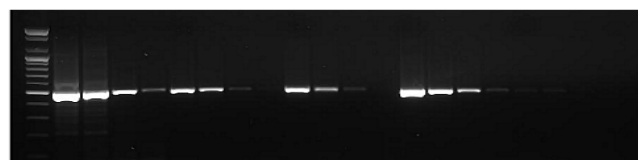
Step	Temperature	Time	Cycles
cDNA synthesis	42~70°C	10~60 min	1
Pre-Denaturation	95°C	5 min	1
Denaturation	95°C	10~30sec	30
annealig	50~65°C	10~30sec	
extension	72°C	1kb/1min	
Final extension	72°C	5min	1

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

Bioneer Q Supplier P Supplier I,V Supplier I,T Supplier  
M 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4



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

**Figure 1. Sensitivity comparison between *AccuPower<sup>®</sup> RocketScript* RT-PCR PreMix and other suppliers' products**

All reactions were carried out following the suppliers' instructions. / Primer set: human myc 495 bp set

## IX. Ordering Information

Cat. No.	Description
K-2501	<i>AccuPower<sup>®</sup> RocketScript</i> RT-PCR Premix, 0.2ml thin-wall 8-Strip tubes with attached cap, 20 µl, 96 tubes
K-2503	<i>AccuPower<sup>®</sup> RocketScript</i> RT-PCR Premix, 0.2ml thin-wall 8-Strip tubes with attached cap, 50 µl, 96 tubes
K-2502	<i>AccuPower<sup>®</sup> RocketScript</i> RT-PCR Premix, 0.2ml thin-wall 8-Strip tubes with attached cap, 20 µl, 480 tubes
K-2504	<i>AccuPower<sup>®</sup> RocketScript</i> RT-PCR Premix, 0.2ml thin-wall 8-Strip tubes with attached cap, 50 µl, 480 tubes