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I. Introduction

AccuPower® RocketScript™ RT-PCR PreMix, RNase H Minus is a ready-to-use, vacuum dried one-step RT-PCR product, containing all components including RocketScript™ Reverse Transcriptase, RNase H Minus for first-strand cDNA synthesis and ProFi Taq DNA polymerase for efficient DNA amplification. RocketScript™ Reverse Transcriptase, RNase H Minus in the premix is a recombinant *M-MLV* reverse transcriptase with enhanced thermostability up to 70°C, as well as eliminated RNase H activity. It is highly efficient in producing full-length cDNA from long RNA transcripts up to 12.5 kb with high degree of secondary structure or high GC content. And, ProFi Taq DNA polymerase in the premix is a unique recombinant Taq DNA polymerase that offers enhanced amplification efficiency and higher fidelity for PCR.

II. Application

- Standard RT-PCR
- Gene expression level analysis
- Virus detection

III. Unit Definition

One unit is defined as the amount of enzyme required to incorporate 1 nmole of deoxynucleotide into acid-precipitable material in 10 min at 37°C using poly A, oligo dT as template primer.

IV. Contents

Component per tube	Amount
RocketScript™ Reverse Transcriptase, RNase H minus	1 U
ProFi Taq DNA polymerase	1 U
Reaction buffer with 1.5 mM MgCl ₂	1 x
dNTP Mixtures (dATP, dCTP, dGTP, dTTP)	Each 300 µM
Stabilizer and tracking dye	Trace

V. Ordering Information

Cat. No.	rxn	Description
K-2231	96 rxns	AccuPower® RocketScript™ RT-PCR PreMix, RNase H Minus, 20 µl, 12 x 0.2 ml thin-wall 8-tube strips with attached cap
K-2232	480 rxns	AccuPower® RocketScript™ RT-PCR PreMix, RNase H Minus, 20 µl, 60 x 0.2 ml thin-wall 8-tube strips with attached cap
K-2233	96 rxns	AccuPower® RocketScript™ RT-PCR PreMix, RNase H Minus, 50 µl, 12 x 0.2 ml thin-wall 8-tube strips with attached cap
K-2234	480 rxns	AccuPower® RocketScript™ RT-PCR PreMix, RNase H Minus, 50 µl, 60 x 0.2 ml thin-wall 8-tube strips with attached cap

VI. Storage

AccuPower® RocketScript™ RT-PCR PreMix, RNase H Minus should be stored at -20°C upon receipt and is stable until the expiration date stated on the label

VII. Specifications

Enzyme	RocketScript™ RTase, RNase H Minus
DNase activity	No
RNase activity	No
RNase H activity	No
Fragment size	Up to 12.5 kb

VIII. Additional Required Materials & Devices

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

IX. 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.

X. Protocol

1. Thaw total RNA, DEPC-water, and primer before use.
2. Add total RNA and specific primer into the AccuPower® RocketScript™ RT-PCR PreMix, RNase H Minus tubes.
3. Add DEPC-water into the AccuPower® RocketScript™ RT-PCR PreMix, RNase H Minus tubes to a total volume of 20 µl (K-2231, K-2232) or 50 µl (K-2233, K-2234). Do not consider small volume of dried pellet in a tube. Following table is a typical example.

Component	Amount	
DEPC-D.W.	X µl	X µl
Specific Primer	10 – 30 pmoles	10 – 30 pmoles
Template RNA	10 pg – 5 µg	10 pg – 5 µg
Total	20 µl	50 µl

4. Dissolve the vacuum dried 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	Cycle
cDNA synthesis	42 - 70°C	30 min	1
Pre-denaturation	95°C	5 min	1
Denaturation	95°C	10 - 30 sec	30-35
Annealing	55°C	10 - 30 sec	
Extension	72°C	1 kb/1 min	
Final extension	72°C	5 min	1

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

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

XI. Notice

Bioneer corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice. All information provided here is subject to change without notice.