[Cat. No.] K-2249

Introduction

AccuPower® RocketScript™ RT Master Mix, RNase H Minus uses *RocketScript™* Reverse Transcriptase (RTase), RNase H Minus independently developed by BIONEER. *RocketScript™* RTase, RNase H Minus is a recombinant *M-MLV* RTase with enhanced thermostability up to 70ºC, 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. In addition, it can synthesize cDNA even with small amounts of 1 pg of total RNA. This product is a ready-to-use mixture for cDNA synthesis including *RocketScript™* RTase, RNase H Minus, reaction buffer, DTT, dNTPs, and RNase inhibitor. It simplifies preparation of reverse transcription reaction mixture by adding template RNA, primers, and nuclease-free water without any extra process.

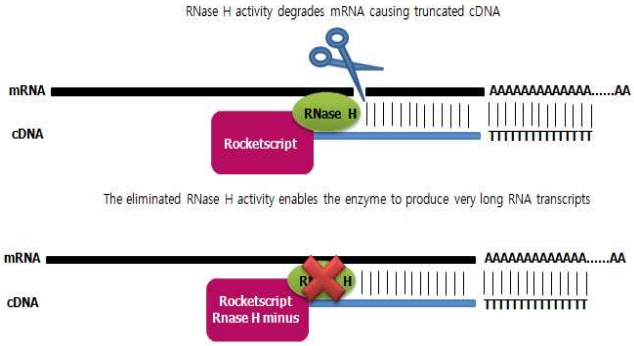

Figure 1. Elimination of RNase H activity. Eliminated RNase H activity from RTase complex enables the RTase to produce longer RNA transcripts, up to 12.5 kb, compared with original *RocketScript™* RTase itself.

Applications

- First-strand synthesis of cDNA from RNA molecules (RT)
- Random priming reaction
- cDNA library construction, Probe labeling
- mRNA 5'–end mapping by primer extension analysis
- PCR, Real-time PCR

Features & Benefits

- Rapid synthesis (9 kb synthesis possible with 10 minutes RT reaction): Time-efficient and economical cDNA synthesis even with secondary structure RNA can be possible by using *RocketScript™* RTase due to excellent thermal stability.
- No RNase H activity: Useful for cDNA synthesis up to 12.5 kb from long target RNA using enzymes developed by proprietary genetic engineering technology.
- Sensitivity: Efficient cDNA synthesis even with small amounts of 1 pg of human total RNA.

Components

Components	Amount
2X Master Mix	1 ml
Oligo dT_{20} (100 pmol/µl)	100 µl
Oligo dN_6 (100 pmol/µl)	100 µl

Composition

2X Master Mix	Concentration
RocketScript™ Reverse Transcriptase, RNase H Minus	200 U
5X Reaction buffer	1X
DTT	0.25 mM
dNTPs (dATP, dCTP, dGTP, dTTP)	Each 250 µM
RNase inhibitors	1 U

Specifications

RocketScript™ Reverse Transcriptase, RNase H Minus	
DNase activity	No
RNase activity	No
RNase H activity	No
Fragment size	Up to 12.5 kb

Storage

Store at -20°C. If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label.

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols

Ordering Information

Description		Cat. No.
1 ml of 2X Master Mix solution	1 ml x 1 ea	K-2249

*** Note:** For Master Mix products, primers (Oligo dT_{20} and Oligo dN_6) are provided in a separate tube.

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.

Explanation of Symbols

LOT Batch Code
Biological Risks
REF Catalog Number
Caution
Consult Instructions For Use
Contains Sufficient for <n> tests
Do not Re-use
Manufacturer
RUO Research Use Only
Temperature Limitation
Use-by Date

Copyright 2022 BIONEER Corporation. All Rights Reserved.

Experimental Procedures

1 Thaw reagents

1. Thaw *AccuPower® RocketScript™* RT Master Mix, RNase H Minus on ice and mix thoroughly before use. Then, briefly spin down components.

2. Dispense appropriate volumes of *AccuPower® RocketScript™* RT Master Mix, RNase H Minus into PCR tubes (not provided). Use 10 µl and 25 µl of 2X Master Mix for 20 µl reaction and 50 µl reaction, respectively.

2 Preparation of reaction mixture

3. Add template RNA and primers into PCR tubes containing *AccuPower® RocketScript™* RT Master Mix, RNase H Minus, and then fill up nuclease-free water to make a total volume of 20 µl or 50 µl.

- Amount of template RNA and primers

Components		20 µl reaction	50 µl reaction
Template RNA	Total RNA	0.01-5 µg	0.01-5 µg
	Poly(A) RNA	0.01-5 µg	0.01-5 µg
Primers	Oligo dT	100 pmol	250 pmol
	Random primer	100 pmol	250 pmol
	Gene specific primer	10-50 pmol	10-50 pmol

4. Mix the reaction mixture by pipetting or vortexing, and briefly spin down.

3 cDNA synthesis

5. Perform the reaction under the following conditions.

5-1. Example 1

Step	Temperature				Time
	dN_6	dN_{12}	dT_{20}	Gene specific	
Primer annealing	15°C	30°C	37°C	Tm of primers	10 min
cDNA synthesis			50°C		30 min
Heat inactivation			95°C		5 min

5-2. Example 2

Step	Temperature	Time
cDNA synthesis	42-70°C	1 hr
Heat inactivation	95°C	5 min

* **Note:** For difficult or high GC-content templates, perform cDNA synthesis at 55°C.

6. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.

Option

- If PCR is needed, transfer 2-5 µl of reaction mixture (synthesized cDNA) into *AccuPower®* PCR PreMix tubes (Cat. No. K-2012, not provided), and perform the reaction under the following conditions.

Step	Temperature	Time	Cycles
Pre-denaturation	95°C	5 min	1 cycle
Denaturation	95°C	20 sec	
Annealing	45-65°C	20 sec	25-35 cycles
Extension	72°C	0.5-1 min/kb	
Final extension	72°C	3-5 min	1 cycle

* **Note:** For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.

Copyright 2022 BIONEER Corporation. All Rights Reserved.