[Cat. No.] K-2235

Introduction

AccuPower® RocketScript™ RT-PCR Master Mix, RNase H Minus is a one-step RT-PCR product that uses *RocketScript™* Reverse Transcriptase (RTase), RNase H Minus and *ProFi Taq* DNA Polymerase 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 can produce efficiently full-length cDNA from long RNA transcripts up to 12.5 kb and synthesize cDNA even with small amounts of 1 pg of total RNA. In addition, included *ProFi Taq* DNA Polymerase provides accurate amplification and higher fidelity for PCR. This product is a ready-to-use mixture for cDNA synthesis and PCR including *RocketScript™* RTase, reaction buffer, DTT, dNTPs, RNase inhibitor, and *ProFi Taq* DNA Polymerase. It simplifies preparation of reverse transcription reaction mixture and PCR mixture by adding template RNA and primers without any extra process.

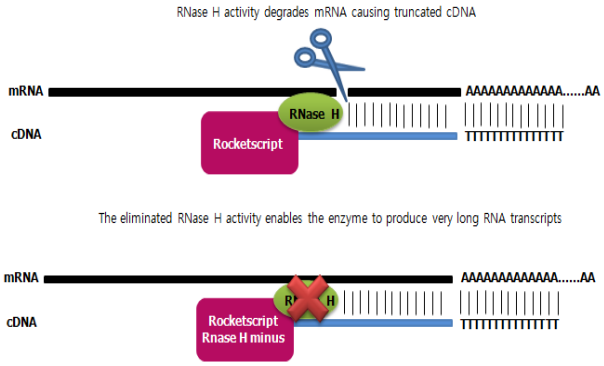


Figure 1. Elimination of RNase H activity. Eliminated RNase H activity from RTase complex enables the RTase to produce longer RNA transcripts, up to 12.5 kb, compared with original *RocketScript™* RTase itself.

Applications

- Standard RT-PCR
- Long kb RT-PCR
- Virus detection
- Gene-expression analysis

Features & Benefits

- Thermostable activity: *RocketScript™* RTase has excellent thermal resistance even at 70°C, so it is suitable for cDNA synthesis of secondary RNA structures.
- 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.
- User-friendly: Simple amplification of long-size RNA difficult to be done with conventional one-step RT-PCR methods.

Composition

Composition	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 inhibitor	1 U
ProFi Taq DNA Polymerase	1 U
Stabilizer and tracking dye	1X

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

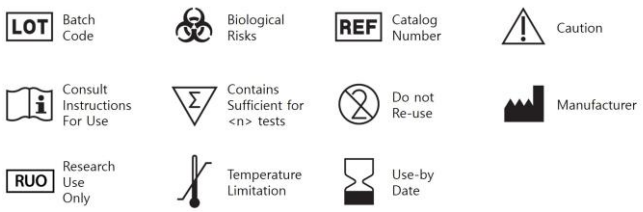
Ordering Information

Description		Cat. No.
1 ml of 2X Master Mix solution	1 ml x 1 ea	K-2235

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.

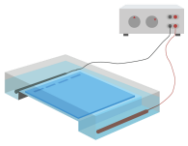
Explanation of Symbols



Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Thaw reagents</td><td>1. Thaw AccuPower® RocketScript™ RT-PCR 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-PCR 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.</td></tr>
<tr><td>2</td><td> 
Preparation of reaction mixture</td><td>3. Add template RNA, primers and nuclease-free water into AccuPower® RocketScript™ RT-PCR Master Mix, RNase H Minus tubes to make a total volume of 20 µl or 50 µl. Do not include the dried pellet.

• Amount of template RNA and primers
<table>
<tr><th colspan="2">Components</th><th>20 µl reaction</th><th>50 µl reaction</th></tr>
<tr><td rowspan="2">Template RNA</td><td>Total RNA</td><td>0.01-5 µg</td><td>0.01-5 µg</td></tr>
<tr><td>Poly(A) RNA</td><td>0.01-5 µg</td><td>0.01-5 µg</td></tr>
<tr><td>Primers</td><td>Gene specific primer</td><td>10-30 pmol</td><td>10-50 pmol</td></tr>
</table>
4. Dissolve the vacuum-dried pellet by pipetting or vortexing, and briefly spin down.</td></tr>
<tr><td>3</td><td> 
RT-PCR</td><td>5. Perform the reaction under the following conditions.
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr>
<tr><td>cDNA synthesis</td><td>42-70°C</td><td>30 min</td><td>1 cycle</td></tr>
<tr><td>Pre-denaturation</td><td>95°C</td><td>5 min</td><td>1 cycle</td></tr>
<tr><td>Denaturation</td><td>95°C</td><td>10-30 sec</td><td></td></tr>
<tr><td>Annealing</td><td>55°C</td><td>10-30 sec</td><td>30-35 cycles</td></tr>
<tr><td>Extension</td><td>72°C</td><td>1 kb/min</td><td></td></tr>
<tr><td>Final extension</td><td>72°C</td><td>5 min</td><td>1 cycle</td></tr>
</table>
* Note: Reaction temperature should be optimized according to Tm value of primers.</td></tr>
<tr><td>4</td><td> 
Analyze with gel electrophoresis</td><td>6. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.

7. Load samples on agarose gel without adding a loading-dye mixture, and perform gel electrophoresis for analysis.</td></tr>
</table>