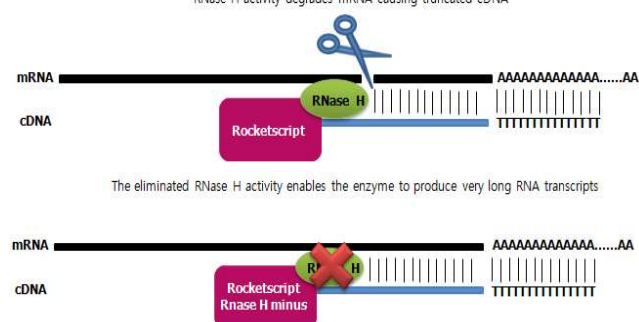


[Cat. No.] Please refer to the **Ordering Information**

Introduction

AccuPower® RocketScript™ RT PreMix, 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 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. In addition, it can synthesize cDNA even with small amounts of 1 pg of total RNA. This product contains vacuum-dried components essential 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.

RNase H activity degrades mRNA causing truncated cDNA



The eliminated RNase H activity enables the enzyme to produce very long RNA transcripts

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.

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

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

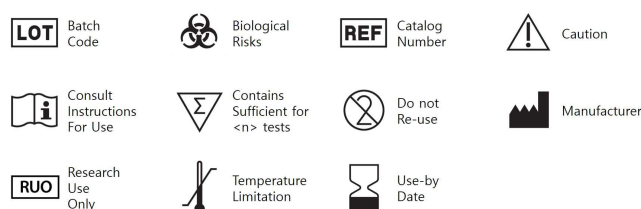
Ordering Information

Description	Cat. No.	
0.2 ml thin-wall 8-tube strips with attached cap	20 μl/rxn K-2221	
	50 μl/rxn K-2223	
	96 tubes	dN ₆ 20 μl/rxn K-2245
		50 μl/rxn K-2246
	dN ₁₂	20 μl/rxn K-2247
		50 μl/rxn K-2248
	dT ₂₀	20 μl/rxn K-2241
		50 μl/rxn K-2243
	480 tubes	20 μl/rxn K-2222
		50 μl/rxn K-2224
		dT ₂₀ 20 μl/rxn K-2242
		50 μl/rxn K-2244




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

Steps		Procedure Details																																								
1	 Preparation of reaction mixture	<p>1. Add template RNA and nuclease-free water into <i>AccuPower® RocketScript™</i> RT PreMix, RNase H Minus tubes to make a total volume of 20 µl or 50 µl. Do not include the dried pellet.</p> <p>* Note: If using a product that does not contain primers (K-2221~K-2224), refer to the following to add primers (not provided).</p> <ul style="list-style-type: none"> Amount of template RNA and primers (K-2221, K-2222, K-2223, K-2224) <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th colspan="2">Components</th> <th>20 µl reaction</th> <th>50 µl reaction</th> </tr> </thead> <tbody> <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 rowspan="3">Primers</td> <td>Oligo dT</td> <td>100 pmol</td> <td>250 pmol</td> </tr> <tr> <td>Random primer</td> <td>100 pmol</td> <td>250 pmol</td> </tr> <tr> <td>Gene specific primer</td> <td>10-50 pmol</td> <td>10-50 pmol</td> </tr> </tbody> </table> <ul style="list-style-type: none"> Amount of template RNA (K-2241, K-2242, K-2243, K-2244, K-2245, K-2246, K-2247, K-2248) <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th colspan="2">Components</th> <th>20 µl reaction</th> <th>50 µl reaction</th> </tr> </thead> <tbody> <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> </tbody> </table>				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	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					
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2	 cDNA synthesis	<p>2. Dissolve the vacuum-dried pellet by pipetting or vortexing, and briefly spin down.</p> <p>3. Perform the reaction under the following conditions.</p> <p>3-1. Example 1</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th rowspan="2">Step</th> <th colspan="4">Temperature</th> <th rowspan="2">Time</th> </tr> <tr> <th>dN₆</th> <th>dN₁₂</th> <th>dT₂₀</th> <th>Gene specific</th> </tr> </thead> <tbody> <tr> <td>Primer annealing</td> <td>15°C</td> <td>30°C</td> <td>37°C</td> <td>T_m of primers</td> <td>10 min</td> </tr> <tr> <td>cDNA synthesis</td> <td></td> <td></td> <td>50°C</td> <td></td> <td>30 min</td> </tr> <tr> <td>Heat inactivation</td> <td></td> <td></td> <td>95°C</td> <td></td> <td>5 min</td> </tr> </tbody> </table> <p>3-2. Example 2</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Step</th> <th>Temperature</th> <th>Time</th> </tr> </thead> <tbody> <tr> <td>cDNA synthesis</td> <td>42-70°C</td> <td>1 hr</td> </tr> <tr> <td>Heat inactivation</td> <td>95°C</td> <td>5 min</td> </tr> </tbody> </table> <p>* Note: For difficult or high GC-content templates, perform cDNA synthesis at 55°C.</p> <p>4. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.</p>				Step	Temperature				Time	dN ₆	dN ₁₂	dT ₂₀	Gene specific	Primer annealing	15°C	30°C	37°C	T _m of primers	10 min	cDNA synthesis			50°C		30 min	Heat inactivation			95°C		5 min	Step	Temperature	Time	cDNA synthesis	42-70°C	1 hr	Heat inactivation	95°C	5 min
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 Option	<ul style="list-style-type: none"> If PCR is needed, transfer 2-5 µl of reaction mixture (synthesized cDNA) into <i>AccuPower®</i> PCR PreMix tubes (Cat. No. K-2012, not provided), and perform the reaction under the following conditions. <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Step</th> <th>Temperature</th> <th>Time</th> <th>Cycles</th> </tr> </thead> <tbody> <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>20 sec</td> <td></td> </tr> <tr> <td>Annealing</td> <td>45-65°C</td> <td>20 sec</td> <td>25-35 cycles</td> </tr> <tr> <td>Extension</td> <td>72°C</td> <td>0.5-1 min/kb</td> <td></td> </tr> <tr> <td>Final extension</td> <td>72°C</td> <td>3-5 min</td> <td>1 cycle</td> </tr> </tbody> </table> <p>* Note: For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.</p>				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														
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