AccuPower® *RocketScript*™ RT Master Mix (V1/2022-03-02)

[Cat. No.] K-2105

Introduction

AccuPower® *RocketScript*™ RT Master Mix can effectively synthesize cDNA with complex secondary RNA structures by using *RocketScript*™ Reverse Transcriptase developed by BIONEER. *RocketScript*™ Reverse Transcriptase originated and engineered from *M-MLV* Reverse Transcriptase can provide increased thermal stability resulting in effective full length first strand cDNA synthesis. It can be used at various temperatures (42-70°C) allowing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptase. This product is a ready-to-use mixture for cDNA synthesis including *RocketScript*™ Reverse Transcriptase, reaction buffer, DTT, dNTPs, and RNase inhibitor. It simplifies preparation of reverse transcription reaction mixture by adding template RNA and primers without any extra process.

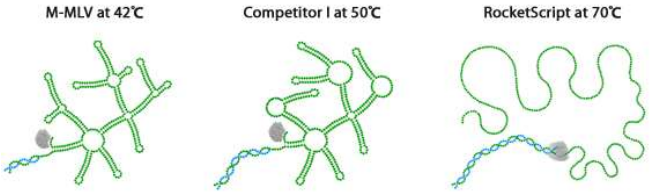


Figure 1. Schematic diagram of 5' UTR of a gene, with complex secondary structure, at three different temperatures.
RocketScript™ Reverse Transcriptase is fully active at 70°C, so it can synthesize complete gene sequences that *M-MLV* and other reverse transcriptase can not synthesize.

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

- Thermostable activity: *RocketScript*™ Reverse Transcriptase has excellent thermal resistance even at 70°C, so it is suitable for cDNA synthesis of secondary RNA structures. The cDNA synthesis temperatures can be set at various temperatures (42-70°C) according to user's experimental conditions, allowing effective cDNA synthesis.
- Stability: Included stabilizer and RNase inhibitor provides high resistance to degradation.
- Reproducibility: Mass production under ISO 9001 quality system allows minimized deviation between lots and reproducible results in replicated tests performed under same conditions and variation.
- Ease-of-use: Reactants are included in a tube, it allows any user simply perform cDNA synthesis by adding template RNA and primers.

Composition

Composition	Concentration
RocketScript™ Reverse Transcriptase	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	
DNase activity	No
RNase activity	No
Fragment size	Up to 10 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

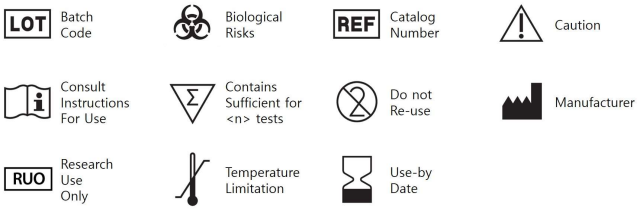
Ordering Information

Description		Cat. No.
1 ml of 2X Master Mix solution	1 ml x 1 ea	K-2105

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



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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 Master Mix on ice and mix thoroughly before use. Then, briefly spin down components.
2. Dispense appropriate volumes of AccuPower® RocketScript™ RT Master Mix 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 and primers into PCR tubes containing AccuPower® RocketScript™ RT Master Mix, and then fill up the nuclease-free water to make a total volume of 20 µl or 50 µl.
• 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 rowspan="3">Primers</td><td>Oligo dT</td><td>100 pmol</td><td>100 pmol</td></tr>
<tr><td>Random primer</td><td>100 pmol</td><td>100 pmol</td></tr>
<tr><td>Gene specific primer</td><td>10-50 pmol</td><td>10-50 pmol</td></tr>
</table>
4. Mix the reaction mixture by pipetting or vortexing, and briefly spin down.</td></tr>
<tr><td>3</td><td> 
cDNA synthesis</td><td>5. Perform the reaction under the following conditions.
5-1. Example 1
<table>
<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>
<tr><td>Primer annealing</td><td>15°C</td><td>30°C</td><td>37°C</td><td>Tm of primers</td><td>10 min</td></tr>
<tr><td>cDNA synthesis</td><td></td><td></td><td>42°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>
</table>
5-2. Example 2
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th></tr>
<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>
</table>
* 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.</td></tr>
<tr><td></td><td> 
Option</td><td>• 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.
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></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>20 sec</td><td rowspan="3">25-35 cycles</td></tr>
<tr><td>Annealing</td><td>45-65°C</td><td>20 sec</td></tr>
<tr><td>Extension</td><td>72°C</td><td>0.5-1 min/kb</td></tr>
<tr><td>Final extension</td><td>72°C</td><td>3-5 min</td><td>1 cycle</td></tr>
</table>
* Note: For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.</td></tr>
</table>

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