AccuPower® *RocketScript*™ Cycle RT Master Mix (V2/2022-03-02)

[Cat. No.] K-2216

Introduction

AccuPower® *RocketScript*™ Cycle RT Master Mix is a next generation cDNA synthesis kit that uses *RocketScript*™ Reverse Transcriptase (*RocketScript*™ RTase) and Cyclic Temperature Reverse Transcription (CTRT) technologies developed by BIONEER. *RocketScript*™ RTase 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. In addition, the CTRT not only can increase the efficiency, but also effective for full-length cDNA synthesis. The CTRT reaction is composed of 2 or 3 steps. The step 1 is performed at 15-25°C, at which short primers are fully annealed. Then, the step 2 is performed at 42-48°C for cDNA synthesis. The step 3 is performed at high temperature 50-55°C at which secondary structure of template RNA obstructing reverse transcription is released. This product is a ready-to-use mixture containing *RocketScript*™ RTase, reaction buffer, DTT, dNTPs, and RNase inhibitors. Primers (Oligo dT_{20} and Oligo dN_6) are provided in separate tubes. It simplifies preparation of reverse transcription reaction mixture by adding template RNA, primers, and nuclease-free water without any extra process.

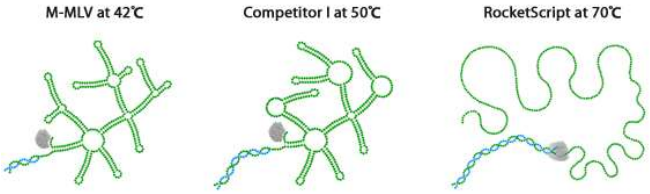

Figure 1. Schematic diagram of 5' UTR of a gene, with complex secondary structure, at three different temperatures.
RocketScript™ Reverse Transcriptase is fully active at 70°C, so it can synthesize complete gene sequences that *M-MLV* and other reverse transcriptase can not synthesize.

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

- Sensitivity & Efficiency: *RocketScript*™ RTase and CTRT technologies provide enhanced sensitivity synthesizing cDNA from low concentration RNA and increased efficiency by applying circulating temperature reaction.
- Thermostable activity: *RocketScript*™ RTase 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.
- Flexible Reaction: As well as CTRT, reverse transcription reaction is possible at a single temperature within 22-55°C.

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

Composition	Concentration
RocketScript™ Reverse Transcriptase	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	
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-2216

*** Note:** For Master Mix products, primers (dT_{20} & 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

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™ Cycle RT Master Mix on ice and mix thoroughly before use. Then, briefly spin down components.
2. Dispense appropriate volumes of AccuPower® RocketScript™ Cycle 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, primers, and nuclease-free water into PCR tubes containing AccuPower® RocketScript™ Cycle RT Master Mix.
• 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>50-100 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>
</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. CTRT reaction (Example 1)
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr>
<tr><td>Primer annealing</td><td>37°C</td><td>10-30 sec</td><td rowspan="3">10 cycles or more</td></tr>
<tr><td>cDNA synthesis</td><td>50°C</td><td>4 min</td></tr>
<tr><td>Melting secondary structure & cDNA synthesis</td><td>55-60°C</td><td>30 sec</td></tr>
<tr><td>Heat inactivation</td><td>95°C</td><td>5 min</td><td>1 cycle</td></tr>
</table>
5-2. CTRT reaction (Example 2)
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr>
<tr><td>Primer annealing</td><td>37°C</td><td>1 min</td><td rowspan="3">10 cycles or more</td></tr>
<tr><td>Melting secondary structure & cDNA synthesis</td><td>42-70°C</td><td>4 min</td></tr>
<tr><td>Heat inactivation</td><td>95°C</td><td>5 min</td><td>1 cycle</td></tr>
</table>
5-3. Single temperature reaction (Example 3)
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr>
<tr><td>cDNA synthesis</td><td>22-55°C</td><td>30-60 min</td><td>1 cycle</td></tr>
<tr><td>Heat inactivation</td><td>95°C</td><td>5 min</td><td>1 cycle</td></tr>
</table>
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>
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