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

[Cat. No.] K-2505

Introduction

AccuPower® *RocketScript*™ RT-PCR Master Mix utilizes one-step RT-PCR performing cDNA synthesis and PCR in a single step, in a single tube. It can effectively synthesize cDNA with complex secondary RNA structures by using *RocketScript*™ Reverse Transcriptase (*RocketScript*™ RTase) 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. This product is a ready-to-use mixture containing *RocketScript*™ RTase, reaction buffer, DTT, dNTPs, RNase inhibitor, and *Top* DNA Polymerase. It simplifies preparation of reverse transcription reaction mixture and PCR mixture by adding template RNA and primers without any extra process. After the reaction, samples can be applied directly on agarose gel for analysis due to included tracking dye.

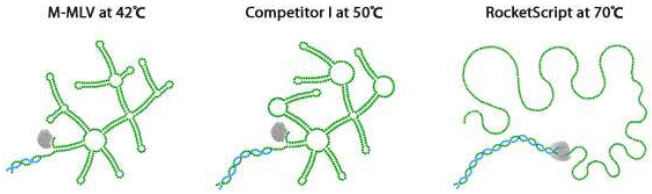


Figure 1. Schematic diagram of 5' UTR of a gene, with complex secondary structure, at three different temperatures.
RocketScript™ Reverse Transcriptase is fully active at 70°C, so it can synthesize complete gene sequences that *M-MLV* and other reverse transcriptase can not synthesize.

Applications

- First-strand synthesis of cDNA from RNA molecules (RT)
- RT-PCR
- cDNA library construction
- Gene expression analysis

Features & Benefits

- Long range RT-PCR: Increased fidelity and accuracy enables one-step RT-PCR of full-length size of target up to 6 kb.
- Sensitivity: Excellent sensitivity and efficiency of cDNA synthesis even with small amount of RNA (10 pg-5 μg).
- 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.
- 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 and PCR in one tube by adding template RNA and primers. In addition, tracking dye and sedimentation agents are included in products for electrophoresis.

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
Top DNA Polymerase	1 U
Stabilizer and tracking dye	1X

Specifications

Top DNA Polymerase	
5' to 3' exonuclease activity	No
3' to 5' exonuclease activity	No
3'–A overhang	Yes
Fragment size	Up to 6 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

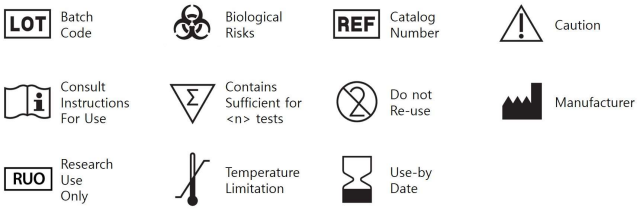
Ordering Information

Description		Cat. No.
1 ml of 2X Master Mix solution	1 ml x 1 ea	K-2505

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.

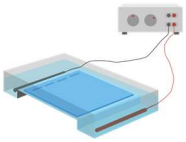
Explanation of Symbols



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 on ice and mix thoroughly before use. Then, briefly spin down components.

2. Dispense appropriate volumes of AccuPower® RocketScript™ RT-PCR 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™ RT-PCR 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>Primers</td><td>Gene specific primer</td><td>10-30 pmol</td><td>10-30 pmol</td></tr></table>
4. Mix the reaction mixture 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>10-60 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>50-65°C</td><td>10-30 sec</td><td>30 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></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>
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