AccuPower® *Dual-HotStart*™ RT-PCR PreMix (V3/2022-03-02)

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

Introduction

AccuPower® *Dual-HotStart*™ RT-PCR PreMix is a one-step RT-PCR product that applied Pyro-HotStart RT technology and HotStart PCR technology to improve problems of non-specific reverse transcription reaction. It can effectively synthesize cDNA with small amounts of template RNA and complex secondary RNA structures. Moreover, it can be applied to various types of samples and provides accurate RT-PCR results with high specificity. This product contains vacuum-dried components including *RocketScript*™ Reverse Transcriptase, reaction buffer, DTT, dNTPs, RNase inhibitor, and HotStart *Taq* DNA Polymerase. It simplifies preparation of reverse transcription reaction mixture and PCR mixture by adding template RNA and primers without any extra process.

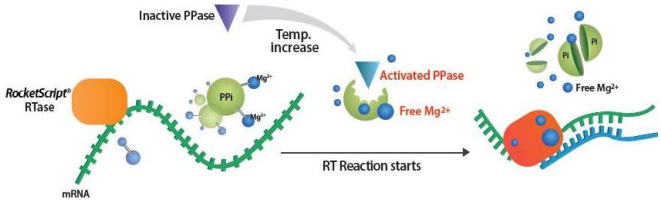


Figure 1. The 1st HotStart reaction. BIONEER's *RocketScript*™ Reverse Transcriptase is completely inhibited by pyrophosphate at temperatures below 50°C. However, *RocketScript*™ RTase becomes fully active at temperatures above 50°C via pyrophosphate hydrolysis with a thermostable pyrophosphatase. This prevents the formation of mis-primed products and primer dimers during the reaction set up process resulting in improved specificity of cDNA synthesis.

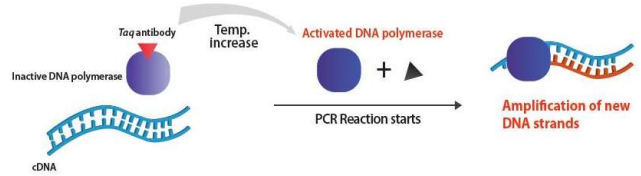


Figure 2. The 2nd HotStart reaction. BIONEER's HotStart *Taq* DNA Polymerase provides superior priming accuracy and specificity that cannot be achieved with other enzymes.

Applications

- Low copy viral/bacterial pathogen load determination in an earlier stage
- Low copy mRNA amplification
- Low copy target RNA quantification
- RNA amplification for microarray and NGS

Features & Benefits

- Sensitivity: Efficient detection even with small amounts of template RNA up to 10 pg of total RNA extracted from cells and tissues.
- Comprehensive applications: Blood and soil samples containing various types of PCR inhibitors can be applied due to its excellent reactivity.
- Specificity: Our premier *Dual-HotStart*™ RT-PCR reaction provides accurate results of target gene amplification by using Pyro-HotStart RT reaction and HotStart PCR.
- Ease-of-use: Reactants are individually packaged in each of the PCR tubes, it allows any user simply perform cDNA synthesis and PCR by adding template RNA and primers.
- 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.

Composition

Composition	Concentration
RocketScript™ Reverse Transcriptase	200 U
5X Reaction buffer	1X
DTT	0.25 mM
dNTPs (dATP, dCTP, dGTP, dTTP)	1.2 mM
RNase inhibitor	1 U
HotStart *Taq* DNA Polymerase	1 U
Stabilizer and tracking dye	1X

Specifications

HotStart *Taq* DNA Polymerase	
5' to 3' exonuclease activity	Yes
3' to 5' exonuclease activity	No
3'–A overhang	Yes
Fragment size	Up to 3 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	96 tubes	20 µl/rxn	K-6710
		50 µl/rxn	K-6711
	480 tubes	20 µl/rxn	K-6712
		50 µl/rxn	K-6713

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

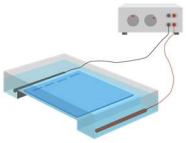
LOT Batch Code · Biological Risks · REF Catalog Number · Caution

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> 
Preparation of reaction mixture</td><td>1. Add template RNA, primers and nuclease-free water into AccuPower® Dual-HotStart™ RT-PCR PreMix 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>10 pg-5 µg</td><td>10 pg-5 µg</td></tr><tr><td>Poly(A) RNA</td><td>10 pg-5 µg</td><td>10 pg-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>
2. Dissolve the vacuum-dried pellet by pipetting or vortexing, and briefly spin down.</td></tr>
<tr><td>2</td><td> 
RT-PCR</td><td>3. 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>10 min</td><td>1 cycle</td></tr><tr><td>Denaturation</td><td>95°C</td><td>10-30 sec</td><td rowspan="4">30 cycles</td></tr><tr><td>Annealing</td><td>50-65°C</td><td>10-30 sec</td></tr><tr><td>Extension</td><td>72°C</td><td>1 kb/min</td></tr><tr><td>Final extension</td><td>72°C</td><td>5 min</td></tr></table>* Note: Reaction temperature should be optimized Tm value of primers.</td></tr>
<tr><td>3</td><td> 
Analyze with gel electrophoresis</td><td>4. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.

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