AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) (V5/2022-03-02)

[Cat. No.] K-6714, K-6715

Introduction

AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) 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, application of uracil DNA glycosylase (UDG) system prevents carryover contaminations. UDG hydrolyzes the N-glycosidic bonds linking uracil and deoxyribose, breaking down the leftover templates inserted with uracil. Before the PCR, contaminants are removed by activating enzymes at 37°C for 2 min and enzymes are deactivated at the higher temperature during the cycles.

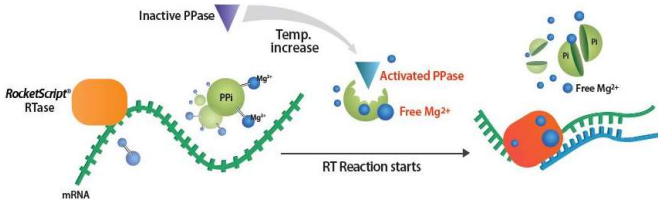


Figure 1. The 1st HotStart reaction. BIONEER's *RocketScript™* Reverse Transcriptase is completely inhibited by pyrophosphate at temperatures below 50°C. However, *RocketScript™* RTase becomes fully active at temperatures above 50°C via pyrophosphate hydrolysis with a thermostable pyrophosphatase. This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

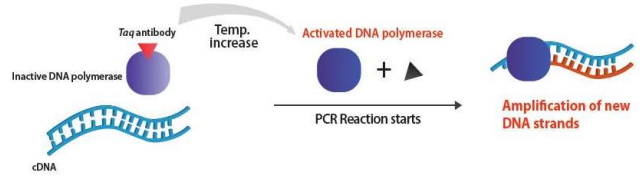


Figure 2. The 2nd HotStart reaction. BIONEER's HotStart *Taq* DNA Polymerase provides superior priming accuracy and specificity that cannot be achieved with other enzymes.

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

- Carryover contamination prevention: Minimized false positives caused by a carryover contamination through application of uracil DNA glycosylase system.
- Specificity: Our premier *Dual-HotStart™* RT-PCR reaction provides accurate results of target gene amplification by using Pyro-HotStart RT reaction and HotStart PCR.

Composition

Composition	Concentration
Uracil DNA glycosylase	1 U
RocketScript™ Reverse Transcriptase	200 U
5X Reaction buffer	1X
DTT	0.25 mM
dNTPs with dUTP	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

Enzyme Inactivation

UDG is inactivated by heating at 95°C for 5 min.

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.

Precautions

UDG activities can be remained after finishing reactions, if it is kept on below 50°C. Therefore, reaction mixture is recommended to freeze immediately after the reaction.

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols

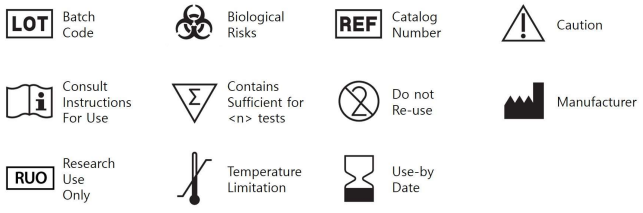
Ordering Information

Description			Cat. No.
0.2 ml thin-wall 8-tube strips with attached cap	96 tubes	20 µl/rxn	K-6714
		50 µl/rxn	K-6715

Notice

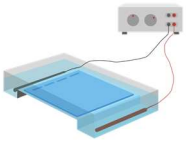
BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols



Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Preparation of reaction mixture</td><td>1. Add template RNA, primers and nuclease-free water into AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) tubes to make a total volume of 20 µl or 50 µl. Do not calculate 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>UDG activation</td><td>37°C</td><td>2 min</td><td>1 cycle</td></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="3">30-40 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><td>1 cycle</td></tr></table>* Note: Reaction temperature should be optimized according to 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>
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