AccuPower® Dual-HotStart™ RT-qPCR PreMix (V3/2022-05-02)

[Cat. No.] K-6704, K-6705, K-6706

Introduction

AccuPower® Dual-HotStart™ RT-qPCR PreMix is a one-step RT-qPCR product based on hydrolysis probe method. It is applied Pyro-HotStart RT technology and HotStart PCR technology to substantially improve the problems of non-specific reactions and enhance the sensitivity. In reverse transcription, enzyme-mediated HotStart method that provides robust, sensitive, and reliable cDNA synthesis results by using BIONEER's *RocketScript*™ Reverse Transcriptase. In real-time PCR, the second HotStart reaction using antibody-based HotStart *Taq* DNA Polymerase provides superior priming accuracy and reduced non-specific reactions such as mis-priming and primer dimer during PCR at a low temperature. This product contains vacuum-dried all components for RT-qPCR, except for template RNA, target-specific primers, and probes. By just adding template RNA, target-specific primers, and probes, reproducible results with high sensitivity and specificity can be obtained. Moreover, this product is compatible with comprehensive types of devices, including *Exicycler*™ 96 and those from other companies, by providing optimized tubes and plates, along with a 2X Master Mix type.

Applications

- Gene expression profiling
- Target RNA quantification
- Microbial detection
- Viral/bacterial pathogen load determination

Features & Benefits

- Specificity: The world's first *Dual-HotStart*™ RT-qPCR reaction provides accurate results of target gene amplification by using Pyro-HotStart RT reaction and HotStart PCR.
- Sensitivity: It can be detected from even a trace amount of template RNA with a wide dynamic range of 10 logs up to 10-10^{10} copies.
- Multiplex: Compatible with many kinds of fluorescent dye (probes) to detect various kinds of target genes.
- Comprehensive template RNA detection: Included *RocketScript*™ RTase can perform reverse transcription at high temperature and even with secondary RNA structures.
- Compatibility: Wide range of samples such as blood and soil containing various PCR inhibitors can be applied due to its excellent reactivity.
- Convenience: Reactants are individually packaged in each of the PCR tubes, it allows any user simply perform cDNA synthesis and real-time PCR by adding template RNA and target-specific primers.
- Stability: Included stabilizer enables enzyme activity to be stable for up to 2 years at -20°C and provides increased stability compared to solution-type products.
- 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.

Components

Components	K-6704	K-6705	K-6706
Tube/Plate	96 tubes	96 tubes	96 tubes
50X ROX dye	-	0.1 ml	-
DEPC-D.W.	1.2 ml x 4 ea	1.2 ml x 4 ea	1.2 ml x 4 ea

*** Note:** ROX dye is used for normalization of intensity by background subtraction. The use of ROX dye is recommended for Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems), but not required for *Exicycler*™ 96 Real-Time PCR System (BIONEER) and CFX96 Real-Time PCR System (Bio-Rad).

Composition

Composition	Concentration
HotStart *Taq* DNA Polymerase	1 U
RocketScirpt Reverse Transcriptase	200 U
10X Reaction buffer with 2 mM $MgCl_2$	1X
10X HotStart buffer	1X
dNTPs (dATP, dCTP, dGTP, dTTP)	1.2 mM

Specifications

HotStart *Taq* DNA Polymerase	
5' to 3' exonuclease activity	Yes
3' to 5' exonuclease activity	No
3'–A overhang	Yes

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

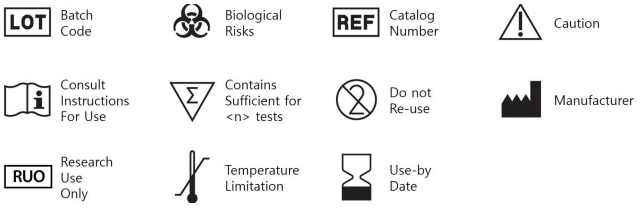
Ordering Information

Description					Cat. No.
Exicycler	8-tube strips	50 µl	optical film included	96 rxn	K-6704
ABI7500	8-tube strips	50 µl	optical film included	96 rxn	K-6705
CFX96	8-tube strips	50 µl	optical film included	96 rxn	K-6706

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, target-specific primers, hydrolysis probe (not provided), 50X ROX dye (optional), and DEPC-D.W. into AccuPower® Dual-HotStart™ RT-qPCR PreMix tubes to make a total volume of 50 µl. Do not include the dried pellet.

• Preparation of reaction mixture
<table><tr><th>Components</th><th>50 µl reaction</th></tr><tr><td>Template RNA (10 pg-100 ng)</td><td>Variable</td></tr><tr><td>Forward primer (10 pmol/µl)</td><td>0.5-5 µl</td></tr><tr><td>Reverse primer (10 pmol/µl)</td><td>0.5-5 µl</td></tr><tr><td>Hydrolysis probe (10 pmol/µl)</td><td>0.5-5 µl</td></tr><tr><td>(Optional) 50X ROX dye</td><td>1 µl</td></tr><tr><td>DEPC-D.W.</td><td>Variable</td></tr><tr><td>Total volume</td><td>50 µl</td></tr></table>* Note: This protocol was validated with the TaqMan® probe as a hydrolysis probe.

2. Seal real-time PCR tubes with adhesive optical sealing film (Cat. No. 3111-4110, provided).

3. Dissolve the vacuum-dried pellet by vortexing, and briefly spin down.</td></tr>
<tr><td>2</td><td> 
RT-qPCR</td><td>4. 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>50-70°C</td><td>15 min</td><td>1 cycle</td></tr><tr><td>Pre-denaturation</td><td>95°C</td><td>3-5 min</td><td>1 cycle</td></tr><tr><td>Denaturation</td><td>95°C</td><td>5-30 sec</td><td rowspan="2">40-45 cycles</td></tr><tr><td>Annealing & Extension</td><td>55-60°C</td><td>30-35 sec</td></tr></table>* Note: Users can adjust the protocol according to their instrument and template sequences to get optimal results.

5. After the reaction is completed, analyze the results.</td></tr>
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