AccuPower® *Dual-HotStart*™ RT-qPCR Master Mix (V0/2022-05-02)

[Cat. No.] K-6707

Introduction

AccuPower® *Dual-HotStart*™ RT-qPCR Master Mix 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 is ready-to-use mixture containing all components for real-time PCR, 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 a 2X Master Mix type, so it is easy to compatible with other company's equipment.

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 a tube, it allows any user simply perform cDNA synthesis and real-time PCR by adding template RNA and target-specific primers and probe.
- 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-6707
2X Master Mix	1.25 ml x 2 ea
10X HotStart buffer	0.5 ml
50X ROX dye	0.1 ml
DEPC-D.W.	1.2 ml

Composition

2X Master Mix	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

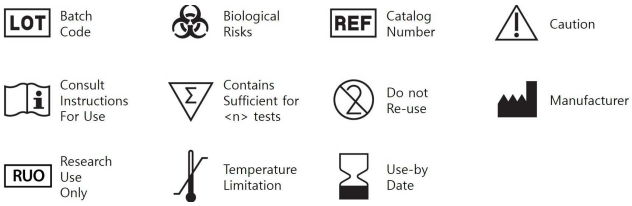
Ordering Information

Description		Cat. No.
2.5 ml of 2X Master Mix solution	1.25 ml x 2 ea	K-6707

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® Dual-HotStart™ RT-qPCR Master Mix on ice and mix thoroughly before use. Then, briefly spin down components.

2. Dispense appropriate volumes of AccuPower® Dual-HotStart™ RT-qPCR Master Mix into PCR tubes (not provided). Use 25 µl of 2X Master Mix for 50 µl reaction.</td></tr>
<tr><td>2</td><td> 
Preparation of reaction mixture</td><td>3. Add template RNA, target-specific primers, hydrolysis probe (not provided), 10X HotStart buffer, 50X ROX dye (optional), and DEPC-D.W. into PCR tubes containing AccuPower® Dual-HotStart™ RT-qPCR Master Mix.

• Preparation of reaction mixture
<table>
<tr><th>Components</th><th>50 µl reaction</th></tr>
<tr><td>2X Master Mix</td><td>25 µl</td></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>10X HotStart buffer</td><td>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

4. Seal real-time PCR tubes with adhesive optical sealing film (Cat. No. 3111-4110, not provided).

5. Mix the reaction mixture by vortexing, and briefly spin down.</td></tr>
<tr><td>3</td><td> 
RT-qPCR</td><td>6. 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.

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