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

[Cat. No.] K-6403

Introduction

AccuPower® *GreenStar*™ RT-qPCR Master Mix is a one-step RT-qPCR product based on intercalating dye method using thermostable reverse transcriptase (RTase) and HotStart PCR technology. By applying BIONEER's *RocketScript*™ RTase which is enhanced thermal stability and processivity, efficient reverse transcription (RT) of RNA molecules with complex secondary structures is possible. In real-time PCR, 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 RT-qPCR, except for template RNA and target-specific primers. By just adding template RNA and target-specific primers, reproducible results with high sensitivity and specificity can be obtained.

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/or for NGS

Features & Benefits

- High sensitivity: Amplification of target gene present in a miniscule amount of 1 pg template RNA.
- High specificity: Minimized experimental errors by non-specific amplification and effective amplification of template RNA existing in a small amount by using HotStart *Taq* DNA Polymerase and thermostable RTase.
- Advanced performance: Comprehensive choice of template RNA for RT-qPCR, even complex secondary structure, by using thermostable *RocketScript*™ RTase, capable of performing RT at high temperature.
- Convenience: Reactants are individually packaged in a tube, it allows any user simply perform one-step RT-qPCR by adding template RNA and target-specific 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.

Components

Components	K-6403
2X Master Mix	1.25 ml x 2 ea
50X ROX dye*	0.1 ml
DEPC-D.W.	1.8 ml

*** 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

2X Master Mix	Concentration
HotStart *Taq* DNA Polymerase	1 U
RocketScript Reverse Transcriptase	200 U
Intercalating dye	0.4X
Reaction buffer with 1.5 mM $MgCl_2$	1X
dNTPs (dATP, dCTP, dGTP, dTTP)	1 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

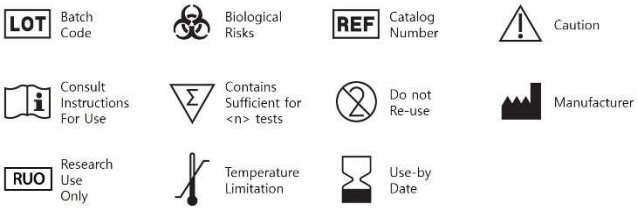
Ordering Information

Description		Cat. No.
2.5 ml of 2X Master Mix solution	1.25 ml x 2 ea	K-6403

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

2. Dispense appropriate volumes of AccuPower® GreenStar™ 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, 50X ROX dye (optional), and DEPC-D.W. into PCR tubes containing AccuPower® GreenStar™ 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>0.5-3 µl</td></tr>
<tr><td>Reverse primer (10 pmol/µl)</td><td>0.5-3 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>
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.
† For cDNA synthesis, it is recommended to start your reaction at least at 50°C, but not exceed at 70°C.

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