AccuPower® GreenStar™ RT-qPCR PreMix
[GreenStar™ RT-qPCR Master Mix] (V2/2018-03-16)

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I. Introduction

AccuPower® GreenStar™ RT-qPCR PreMix and Master Mix detects desired target genes precisely from various samples. This real-time PCR reagent using dsDNA binding dye-based product measures gene amplification through fluorescence evaluation at each cycle.To start your reaction, simply add template RNA and specific primers to your gene of interest into a reaction vessel containing the mastermix or lyophilized premix. It has a feature of selective synthesis of target cDNA from tiny amount of template RNA, and is useful for viral RNA detection or for quantitative gene expression.

II. Application

- 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

III. Principle

1) *RocketScript*™ Reverse Transcription

RocketScript™ Reverse Transcriptase in the *AccuPower® GreenStar*™ RT-qPCR PreMix is genetically engineered *M-MLV* reverse transcriptase with enhanced thermal stability and outstanding processivity. The enzyme also features increased specificity and improved efficiency allowing efficient reverse transcription of RNA molecules with complex secondary structures.

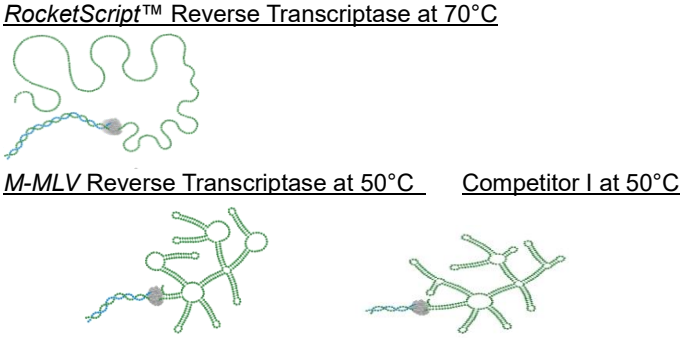

Figure1. Schematic representation of the 5'UTR of a gene, with complex secondary structure, at two different temperatures. Note that *RocketScript* shows full activity at 70°C allowing it to synthesize the complete gene sequence where *M-MLV* and other Reverse Transcriptases fail.

2) HotStart Polymerase Chain Reaction

Bioneer's HotStart *Taq* DNA polymerase provides superior priming accuracy and specificity that cannot be achieved with other enzymes. You will use less enzyme per reaction, save money, and get higher sensitivity than with other hotstart enzymes.

IV. Storage

For long term storage, *AccuPower® GreenStar*™ RT-qPCR PreMix should be stored at -20°C upon receipt and is stable until the expiry date stated on the label.

V. Specifications

Enzyme	*RocketScript*™ RTase + Hotstart *Taq*
DNase activity	No
RNase activity	No
5' to 3' exonuclease	Yes
3' to 5' exonuclease	No
3'-A overhang	Yes
Fragment Size	Up to 200 bp

VI. General precautions

- Wear gloves during experiments to prevent contamination.
- Store positive materials, such as samples and control templates, in a separate freezer from the kit.
- Add templates to the reaction mixture in a hood.

VII. Additionally Required Materials & Devices (Not provided in this kit)

- Thermal Cycler for real-time PCR
- Target-specific primers
- Calibrated micropipette
- Vortex mixer
- Sterilized micropipette tips with filters
- Optical adhesive films for real-time PCR
- High-speed Centrifuge with rotors for microtiter plates

VIII. Content

Cat. No	Descriptions
K-6400	*AccuPower® GreenStar*™ RT-qPCR PreMix, 50 µl/rxn, 8-well strips, 96 rxn, *Exicycler*™ 96, optical film included
K-6403	*AccuPower® GreenStar*™ RT-qPCR Master Mix (2X), 2.5 ml (1.25 ml x 2)

Cat. No	Kit Contents
K-6400	8-well strip x 12 strips DEPC-D.W. 1.8 ml x 4 tube
K-6403	2X Master Mix 1.25 ml x 2 tubes DEPC-D.W. 1.8 ml x 1 tube * ROX dye (50X) 0.1 ml x 1 tube

*Customized Service: *AccuPower® GreenStar*™ RT-qPCR PreMix can be provided in PCR tubes from several instruments. Please contact Bioneer for further detail.

*ROX dye is used for normalization of light intensity by background subtraction. The use of that dye (50X) is recommended for the Applied Biosystems Real-Time PCR System, but for neither *Exicycler*™ 96 nor Bio-Rad CFX qPCR system. Follow manufacturer's recommendations regarding reference dyes.

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IX. Protocol

1. Thaw total RNA, DEPC-water, and primer before use.
2. Add following RT-PCR reagents into *AccuPower® GreenStar™* RT- qPCR PreMix tube (per reaction).

PreMix (K-6400)	
Components	**Amount**
RT-PCR Forward-Primer*	1 to 30 pmole
RT-PCR Reverse-Primer*	1 to 30 pmole
Template	10 pg to 100 ng
DEPC-distilled water.	Adjust to final volume

2X Master Mix (K-6403)	
Components	**Amount**
2X Master Mix	1X
RT-PCR Forward-Primer*	1 to 30 pmole
RT-PCR Reverse-Primer*	1 to 30 pmole
Template	10 pg to 100 ng
(Optional) 50X ROX dye	1X final concentration
DEPC-distilled water.	Adjust to final volume

* ***The primer should be designed according to the following suggestions;***
Primer length: 17~25 mer
GC content of primer: 40-60%
Target length: ≤ 200 bp (optimally, 80-150 bp)
Recommend Tm: between 58–65°C
Avoid any GC-rich or AT-rich sequence at the 3' end.

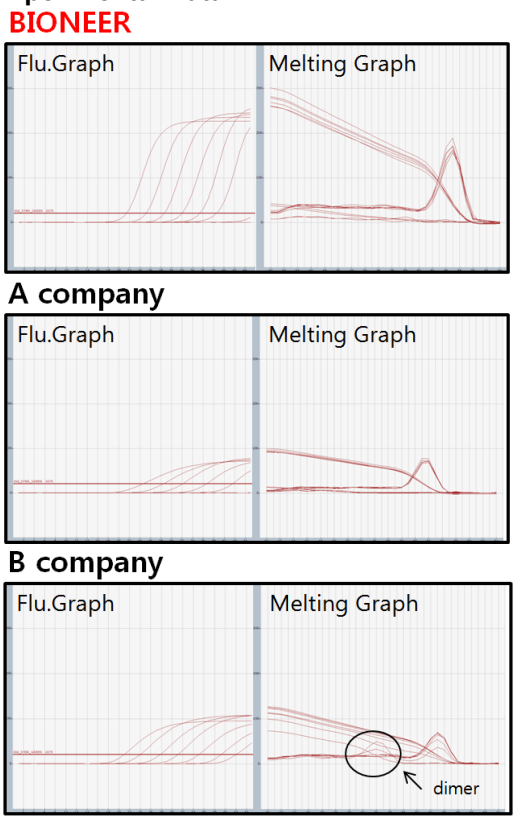
3. Seal the tubes or plate(s) using optical adhesive film for real-time PCR or optically clear cap strips.
4. Completely mix by vortexing (or by pipetting up and down several times before sealing the tubes or plate(s)).
5. Centrifuge at 3,000 rpm, for 2 min (optional – necessary only if mixing was performed by vortexing).
6. Load the tube or plate onto your Real-time PCR instrument.
7. Perform the reaction under the following conditions*:

Step	Condition	Cycle
Reverse transcription	50-70°C, 15 min	1
Pre-Denaturation	95°C, 3-5 min	1
Denaturation	95°C, 5-30 sec	40-45
Annealing/Extension /Detection	55-60°C, 5-30 sec	
Melting		1

8. It is recommended to start your reaction at least at 50°C, but not exceed at 70 °C, for the RT portion of the experiment.
9. After reaction is completed, perform data analysis.

**** This recommended protocol can be modified to get the optimal results, based on the real-time PCR instrument and target RNA sequences.***
**** For 3-step cycling protocols, annealing temperature should be set at primer's Tm-5°C for 5~30 sec. Optimal extension condition is 72°C for 30 sec.***

X. Experimental Data



RNA (ng)	BIONEER	A company	B company
100	21.43	24.73	21.04
10	25.18	28.50	24.96
1	27.62	32.18	27.80
0.1	31.07	36.66	31.14
0.01	35.40	41.59	35.11
0.001	38.58	UD	39.29
NTC	UD	UD	42.12

Figure 2. Comparison of specificity between *AccuPower® GreenStar™* RT-qPCR PreMix and other companies' master mixture.
*NTC: No template control
*UD: Undetected

XI. 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. All information provided here is subject to change without notice.

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