AccuPower® *RocketScript*™ RT-qPCR PreMix, MasterMix (2X)

I. Introduction

AccuPower® *RocketScript*™ RT-qPCR PreMix is a ready-to-use reagent containing all components necessary for real-time RT-PCR, except for template, target-specific primers and fluorogenic probe. To start your reaction, simply add template RNA, primers and probe specific to your gene of interest into a reaction vessel containing the MasterMix or PreMix. The 2 x MasterMix is stable for one-year at -20°C, and lyophilized PreMix is stable for 2-years at -20°C.

This product is ideal for use in hydrolysis probe-based real-time PCR experiments for the amplification and detection of RNA: e.g. differential gene expression profiling, SNP (Single Nucleotide Polymorphism) analysis, and evaluation of RNAi products. This product provides reproducible results with superior specificity, high sensitivity, wide dynamic range and accurate quantification.

II. Principle

AccuPower RocketScript RT-qPCR PreMix uses a dual Hotstart RT-qPCR technique that detects only the desired target gene.

1) Hostart Reverse Transcripton

The AccuPower RocketScript RT-qPCR PreMix uses a unique enzyme-mediated HotStart method that provides robust, sensitive, and reliable cDNA synthesis results. Bioneer's RocketScriptTM reverse transcriptase is completely inhibited by pyrophosphate at temperatures below 50°C. However, RocketScript reverse transcriptase 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.

2) Hotstart Polymerase Chain Reaction

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

III. Content

Cat. No	Size	Descriptions
K-6700	96 tests	*AccuPower RocketScript* RT- qPCR PreMix, *Exicycler*™ 96, 12 strips, *Exicycler* 8-well strip, 50μℓ/rxn, optical film included
K-6701	96 tests	*AccuPower RocketScript* RT- qPCR PreMix, ABI7500, 12 strips, ABI7500 8-well strip, 50 μℓ/rxn, optical film included
K-6702	96 tests	*AccuPower RocketScript* RT- qPCR PreMix, Opticon, 12 strips, Opticon 8-well strip, 50 μℓ/rxn, optical film included
K-6703	100 tests	*AccuPower RocketScript* RT- qPCR Master Mix (2X)

Cat. No	Kit Contents
K-6700	8-well strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes
K-6701	8-well strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes * ROX dye (50X) 0.1 ml x 1 tube
K-6702	8-well strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes * ROX dye (50X) 0.1 ml x 1 tube
K-6703	2X Master Mix 0.625ml x 4 tubes DEPC-D.W. 1.2 ml x 1 tubes * ROX dye (50X) 0.1 ml x 1 tube

* Customized Service: *AccuPower RocketScript* RT-qPCR PreMix can provide in various instrument's PCR tube.

* ROX dye is used for normalization of light intensity by background subtraction. The use of ROX dye (50X) is recommended for the Applied Biosystems 7500 Real-Time PCR System.

* The use of ROX dye is not required for Bioneer *Exicycler* 96 and Bio-Rad DNA engine Opticon. Follow manufacturer's recommendations regarding reference dyes.

IV. Storage

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

V. Additionally Required Materials & Devices

- Thermal Cycler for real-time PCR (authorized instruments)
- Target-specific primers and TaqMan-based probe
- Calibrated micropipette, Sterilized micropipette tips with filters
- Optical adhesive films for real-time PCR
- High-speed Centrifuge with rotors for microtiter plates
- Vortex mixer, Desktop centrifuge, Disposable powder-free gloves

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 or a spatially separated facility.

VII. Protocol

1. Add following RT-PCR reagents into *AccuPower RocketScript* RT-qPCR PreMix tube (per reaction)

   * Customized Service: *AccuPower RocketScript* RT-qPCR PreMix can provide in various instrument's PCR tube.

PreMix (K-6700, K-6701, K-6702)	
Components	**Final Concentration**
RT-PCR Forward-Primer	5 to 50 pmole
RT-PCR Reverse-Primer	5 to 50 pmole
TaqMan® Probe	5 to 50 pmole
Template	10 pg to 100 ng
(Optional) 50X ROX dye	1 X
DEPC-distilled water.	Adjust to final volume

2X Master Mix (K-6703)	
Components	**Final Concentration**
2X Master Mix	1 X
RT-PCR Forward-Primer	5 to 50 pmole
RT-PCR Reverse-Primer	5 to 50 pmole
TaqMan® Probe	5 to 50 pmole
Template	10 pg to 100 ng
(Optional) 50X ROX dye	1 X
DEPC-distilled water.	Adjust to final volume

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

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, 30-35 sec	

7. It is recommended to start your reaction at 50 °C for the RT portion of the experiment.
8. 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 DNA sequences***.

VIII. Experimental Example

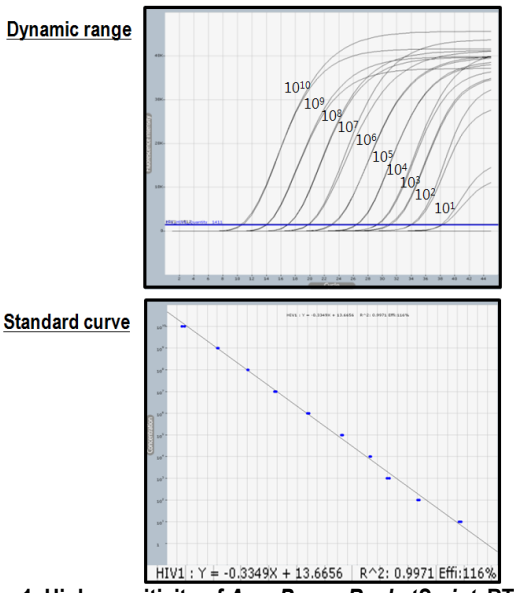


Figure 1. High sensitivity of *AccuPower RocketScript* RT-qPCR PreMix.
Experiment with HIV target. 10 fold serial dilution of Template RNA. (10^{10} copies ~ 10 copies)

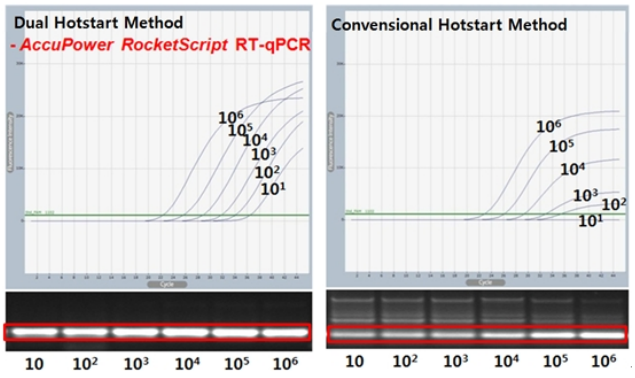
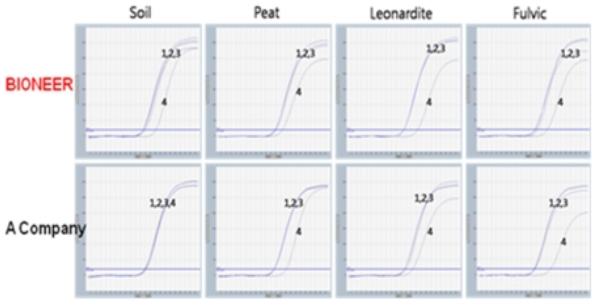


Figure 2. High specificity of *AccuPower RocketScript* RT-qPCR PreMix.
Experiment with HCV target. 10 fold serial dilution of Template RNA (10^6 copies ~ 10 copies) spiked in Human Total RNA. *AccuPower RocketScript* RT-qPCR PreMix using dual Hotstart RT-qPCR method accurately amplifies target RNA without non-specific amplification, even at low concentration of template.

a)

PCR inhibitor		Bioneer	A company
		Totally inhibition (PPM)	
Humic acid	Soil	10,000	*
	Peat	10,000	10,000
	Leonardite	10,000	10,000
	Fulvic	10,000	10,000

* : No inhibition



b)

PCR Inhibitor	Bioneer	A company
	Totally inhibition 농도 (PPM)	
Hemoglobin	*	*
Blood-EDTA	1,000	100

* : No inhibition

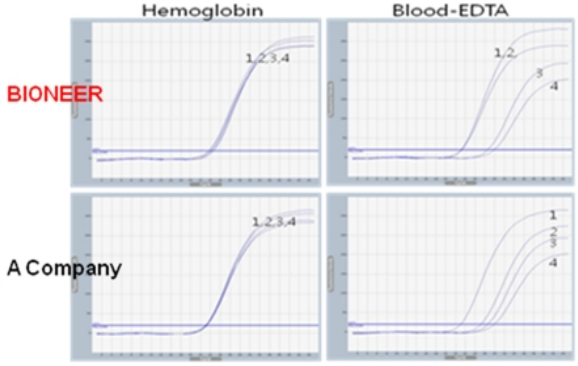


Figure 3. PCR inhibitor (a; Humic acid, b; Hemoglobin, EDTA) study using *AccuPower RocketScript* RT-qPCR PreMix