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I. Introduction

AccuPower® Dual-Hotstart RT-PCR PreMix is a ready-to-use reagent containing all components necessary for RT-PCR except for template and target-specific primers. To start your reaction, simply add template RNA and primers specific to your gene of interest into a reaction vessel containing the vacuum dried PreMix. The PreMix is stable for 2 years at -20°C.

II. Principle

AccuPower Dual-Hotstart RT-PCR PreMix uses a *Dual HotStart* RT-PCR technique that detects only the desired target gene.

1) HotStart Reverse Transcription

The *AccuPower Dual-Hotstart* RT-PCR PreMix uses a unique enzyme-mediated *HotStart* method that provides robust, sensitive, and reliable cDNA synthesis results. Bioneer's *RocketScript™* 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 cannot be achieved with other enzymes. You will use less enzyme per reaction, save money, and get higher sensitivity than with other hotstart enzymes.

III. Storage

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

IV. Content

Cat. No	Size	Descriptions
K-6710	96 tests	<i>AccuPower Dual-Hotstart</i> RT-PCR PreMix, 0.2ml thin-wall 8-Strip tubes with attached cap, 20 µl/rxn
K-6711	96 tests	<i>AccuPower Dual-Hotstart</i> RT-PCR PreMix, 0.2ml thin-wall 8-Strip tubes with attached cap, 50 µl/rxn
K-6712	480 tests	<i>AccuPower Dual-Hotstart</i> RT-PCR PreMix, 0.2ml thin-wall 8-Strip tubes with attached cap, 20 µl/rxn
K-6713	480 tests	<i>AccuPower Dual-Hotstart</i> RT-PCR PreMix, 0.2ml thin-wall 8-Strip tubes with attached cap, 50 µl/rxn

V. 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.

VI. Additional Required Materials & Devices

- Thermal Cycler for PCR (authorized instruments)
- Target-specific primers
- Calibrated micropipette
- Vortex mixer
- Sterilized micropipette tips with filters
- High-speed Centrifuge with rotors for microtiter plates

VII. Protocol

[20 µl reaction volume]

1. Thaw Total RNA, DEPC-water and Specific primer before use.
2. Add Total RNA and Specific primer into *AccuPower Dual-Hotstart* RT-PCR PreMix tubes.

Components		Amount
Template RNA	Total RNA	10pg ~5µg
	Poly(a) RNA	10pg ~5µg
Specific Primer		10~30 pmoles

3. Add DEPC-water into *AccuPower Dual-Hotstart* RT-PCR PreMix tubes to a total volume of 20 µl. Do not calculate the dried pellet.
4. Dissolve the vacuum dried pellet by flick with your finger or pipetting, and briefly spin down.
5. Perform the reaction under the following conditions.

Step	Temperature	Time	Cycles
cDNA synthesis	42~70 °C	10~60 min	1
Pre-Denaturation	95 °C	10 min	1
Denaturation	95 °C	10~30sec	30
Annealing	50~65 °C	10~30sec	
Extension	72 °C	1kb/1min	
Final extension	72 °C	5min	1

6. Maintain the reaction at 4°C after amplification, the sample can be stored at -20°C until use

Note: reaction temperature should be selected to fit the Tm value of Primers

VIII. Experimental data

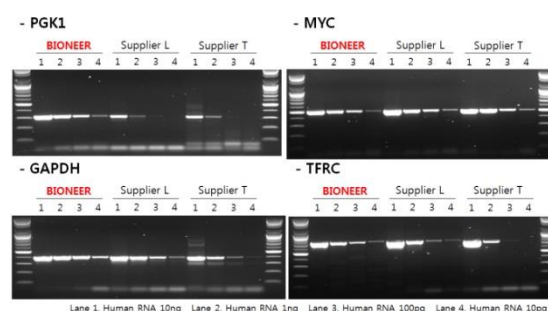


Figure 1. Sensitivity comparison between *AccuPower Dual-Hotstart* RT-PCR PreMix and other suppliers' products

IX. 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.