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I. Introduction

AccuPower® Hotstart *Pfu* PCR PreMix is a ready-to-use vacuum-dried master mix containing all components for high fidelity PCR. Just addition of primers and template into the tube provides reproducible results. *AccuPower®* Hotstart *Pfu* PCR PreMix uses a unique enzyme-mediated Hotstart PCR method that reduces pre-PCR misprimings, primer dimers, artifacts, and any other non-specific amplification. Besides *AccuPower®* Hotstart *Pfu* PCR PreMix provides sensitivity, high specificity and proofreading activity. So you'll get fewer errors in your PCR product.

II. Application

- Gene cloning with blunt ends
- Site-directed mutagenesis
- High fidelity amplification
- High specificity PCR
- cDNA amplification

III. Contents

Component	Concentration
Pfu DNA polymerase	1 U
dNTP (dATP, dCTP, dGTP, dTTP)	Each 250 μM
Reaction Buffer, with 1.5mM MgCl ₂	1X
Stabilizer and tracking dye ¹⁾	

IV. Principle

Enzyme-mediated Hotstart PCR method

AccuPower® Hotstart *Pfu* PCR PreMix is designed for HotStart PCR to provide higher PCR specificity by use of pyrophosphatase and pyrophosphate. Pyrophosphate (PPi) has high affinity for Mg²⁺ ion, which is essential for PCR reaction. The binding of PPi to Mg²⁺ inhibits pfu DNA polymerase activity. This prevents the formation of misprimed products and primer-dimers at low temperature. Pyrophosphatase is activated above 70°C and hydrolyzes PPi to Pi Which then releases Mg²⁺ to activate Taq DNA polymerase. Thus, it increases PCR efficiency and provides high PCR specificity.

V. Features and Benefits

- **High fidelity:** *AccuPower®* Hotstart *Pfu* PCR PreMix has the high fidelity (error rate = 1.9×10^{-6}) that it will reduce the mismatching during DNA amplification.
- **High specificity:** Pyrophosphate (PPi) has high affinity for Mg²⁺. By adding PPi to the reaction mixture, the Mg²⁺ ions necessary for normal PCR is bound thus preventing DNA polymerase activity. This PPi-Mg²⁺ binding prevents non-specific before PCR (zero-cycle) product formation. Upon thermal cycling, the pyrophosphatase (PPase) that is also added to the mixture is activated (>70 °C) and hydrolyzes the PPi to 2 phosphate groups and facilitates the release of Mg²⁺, which is then available for DNA polymerase to use and resume normal activity
- **Easy-to-use:** Just add template and primers and start your reaction. dNTPs, buffer and enzyme are provided
- **Reproducibility:** Bioneer's strict quality controlled production system ensures that your results will be reproducible experiment after experiment.

VI. Additional Required Materials & Devices

- Thermal cycler for PCR
- Target-specific primers, Template DNA
- Calibrated micropipette
- Sterilized micropipette tips

VII. General Precautions

- Wear gloves during experiments to prevent contamination.
- Store positive materials, such as samples and control templates, in separated freezer from freezers for the kit
- Add templates to the reaction mixture in clean bench or a spatially separated facility

VIII. Storage

AccuPower® Hotstart *Pfu* PCR PreMix should be stored at -20°C upon receipt, and is stable until the expiration date stated on the label.

X. Notice to Purchaser

AccuPower® Hotstart *Pfu* PCR PreMix provides sensitivity, high specificity and proofreading activity. Bioneer corporation reserve 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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XI. Protocol

1. Thaw template DNA, and primer before use.
2. Add template DNA and primer into the *AccuPower®* Hotstart Pfu PCR PreMix tubes or plates.

• Recommended amount of template and primer

Components	20 µl reaction	50 µl reaction
Template DNA	1–500 ng	1–500 ng
Forward primer (10 pmol/µl)	0.5-2 µl	1-5 µl
Reverse primer (10 pmol/µl)	0.5-2 µl	1-5 µl

3. Add distilled water into the *AccuPower®* Hotstart Pfu PCR PreMix tubes to a total volume of 20 µl or 50µl. Do not calculate any volume for the dried pellet.
4. Dissolve the vacuum-dried Blue pellet completely and spin down either by using Bioneer's ExiSpin Vortex /Centrifuge or by pipetting up and down for several times and then briefly spinning down.
5. Perform the reaction under the following conditions.

•PCR cycling condition

Step	Temperature	Time	No. of Cycles
(optional)	60°C	1mim	1
Pre-denaturation	95°C	5 min	1
Denaturation	95°C	30 sec	30~35
Annealing	55°C	30 sec	
Extension	72°C	1 min/kb	
Final-Extension	72°C	5 min	1

*note: pre-incubation step is necessary to allow the hotstart

- component to hydrolyze
6. Maintain the reaction at 4°C after the completion of amplification. The sample is recommended to be stored at -20 °C until use.
7. Load 5µl of the reaction mixture directly on agarose gel without adding a loading dye to analyze the PCR products

XIII. Ordering Information

Cat. No.	Description
K-2301	<i>AccuPower®</i> Hotstart Pfu PCR PreMix, 0.2ml thin-wall tubes with attached cap, 20 µl reaction, 96 tubes
K-2302	<i>AccuPower®</i> Hotstart Pfu PCR PreMix, 0.2ml thin-wall tubes with attached cap, 50 µl reaction, 96 tubes
K-2303	<i>AccuPower®</i> Hotstart Pfu PCR PreMix, 0.2ml thin-wall tubes with attached cap, 20 µl reaction, 480 tubes
K-2304	<i>AccuPower®</i> Hotstart Pfu PCR PreMix, 0.2ml thin-wall tubes with attached cap, 50 µl reaction, 480 tubes

Visit us at www.bioneer.com to learn about Bioneer products and services.

XII. Experimental Data

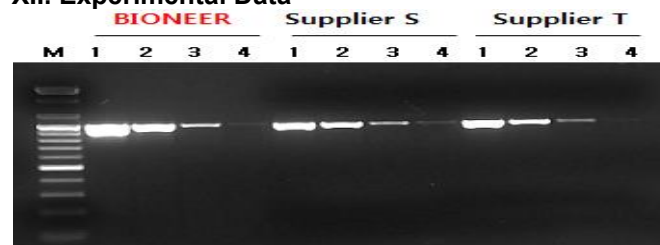


Figure1. Comparison of PCR amplification efficiency between *AccuPower* Hotstart Pfu PCR PreMix from Bioneer and other suppliers' PCR master mix. PCR reactions were performed following the supplier's protocols, using 10ng,1ng,100pg and 10pg Human genomic DNA.

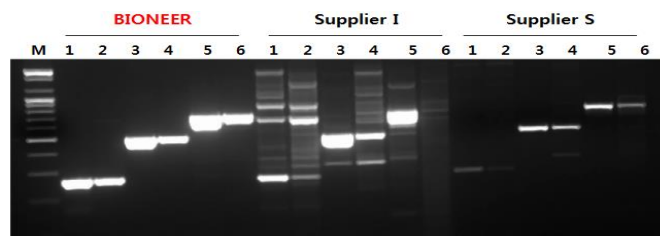


Figure2. Comparison of PCR amplification specificity between *AccuPower* HotStart pfu PCR PreMix from Bioneer and other suppliers' pfu DNA polymerase. Three different primer-template systems were amplified under the same conditions.

Lane M: 100 bp DNA Ladder (Bioneer, Cat. No. D-1030)
Lane 1 ,2: 100 ng, 10 ng DNA, ApoE primer set (268 bp)
Lane 3, 4: 100 ng, 10ng DNA, PrP primer set (500 bp)
Lane 5, 6: 100 ng, 10ng DNA, PrP primer set (705 bp)

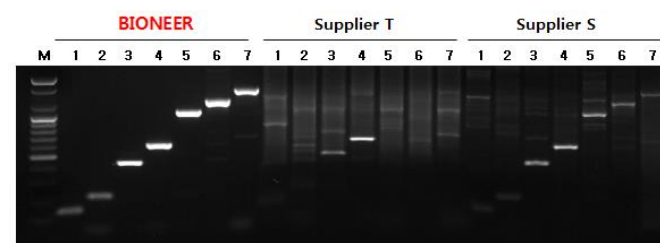


Figure3. *AccuPower* Hotstart Pfu PCR PreMix has high amplification efficiency and specificity. Specificity test was performed using 7 different sets of primers targeting the P53 gene. 10 ng of human genomic DNA was used for each PCR reaction.