AccuPower® PyroHotStart Taq PCR PreMix

(V2/2018-04-12)

Bioneer Corporation
8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302
Republic of Korea
Tel: +82-42-930-8777 (Korea : 1588-9788)
Fax: +82-42-930-8688 E-mail: sales@bioneer.com

Bioneer Inc.
155 Filbert St. Suite 216
Oakland, CA 94607, USA
Toll Free: +1-877-264-4300 Fax: +1-510-865-0350
E-mail: order.usa@bioneer.us.com

Bioneer R&D Center
Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro
Bundang-gu, Seongnam-si, Gyeonggi-do, 13488
Republic of Korea
Tel: +82-31-628-0500 Fax: +82-31-628-0555

I. Introduction

AccuPower® PyroHotStart Taq PCR PreMix is a convenient vacuum-dried PCR master mix containing *PyroHotStart Taq* DNA polymerase, dNTPs, reaction buffer, tracking dye, and patented stabilizer and is aliquoted in 8-strip PCR tubes. The premix utilizes a unique, innovative enzyme-mediated HotStart method that provides robust, sensitive, and reliable PCR results. Bioneer's *Taq* DNA Polymerase is completely inhibited by pyrophosphate at temperatures below 70°C. However, *Taq* DNA polymerase becomes fully active at temperatures above 70°C via pyrophosphate hydrolysis with a thermostable pyrophosphatase. This prevents the formation of mis-primed products, as well as primer-dimers, during the reaction set up process resulting in improved specificity.

In addition, *AccuPower® PyroHotStart Taq* PCR PreMix makes hot-start PCR simple and easy, eliminating the extra handling steps and contamination risks associated with conventional hot-start methods.

II. Application

- High specificity PCR
- High sensitivity PCR
- Low-copy target PCR
- Multiple primer pairs PCR
- cDNA template PCR
- T-A cloning

III. Contents

Components	*Final Concentration*
PyroHotStart Taq DNA polymerase	*1 U*
dNTP Mixture (dATP, dCTP, dGTP, dTTP)	*Each 250 μM*
Reaction buffer, with 1.5 mM $MgCl_2$	*1 X*
Stabilizer and tracking dye[1)]	*1 X*

1) *AccuPower® PyroHotStart Taq* PCR PreMix is premixed with Xylene Cyanol. Xylene Cyanol migrates at approximately 4 kb on a 1% agarose gel.

IV. Principle

AccuPower® PyroHotStart Taq PCR PreMix is designed for HotStart PCR to provide higher PCR specificity by use of pyrophosphatase and pyrophosphate. Pyrophosphate (PPi) has high affinity for Mg^{2+} ion, which is essential for PCR reaction. The binding of PPi to Mg^{2+} inhibits *Taq* DNA polymerase activity. This prevents the formation of mis-primed products and primer-dimers at low temperature.

Pyrophosphatase is activated above 70℃ and hydrolyzes PPi to Pi which then releases Mg^{2+} to activate *Taq* DNA polymerase. Thus, it increases PCR efficiency and provides high PCR specificity.

V. Storage

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

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 a separate freezer from the kit.
- Add templates to the reaction mixture in a hood or a spatially separated facility.

VIII. Protocol

1. Thaw template DNA, and primer before use.
2. Add template DNA and primer into the *AccuPower® PyroHotStart Taq* 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® PyroHotStart* Taq PCR PreMix tubes to a total volume of 20 μl (K-2611, K-2612) or 50 μl (K-2613, K-2614). 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 (15 second vortex on high followed by 5 second spins at 1,500 rpm – x 4 cycles) or by pipetting up and down for several times and then briefly spinning down.
5. Perform the reaction under the following conditions.

• In case of Standard PCR

Step	Temp.	Time	Cycles
Pre-denaturation	95 °C	5 min	1 cycle
Denaturation	95 °C	15-30 sec	25~35 cycles
Annealing	45-65 °C	15-30 sec	
Extension	72 °C	1 min/kb	
Final extension	72 °C	Optional. Normally 3~5 min	1 cycle

Note: The optimal annealing temperature depends on the melting temperature of the primers.

• In case primers Tm value is higher than 65 °C or PCR product size is bigger than 5 kb.

Step	Temp.	Time	Cycles
Pre-denaturation	95 °C	5 min	1 cycle
Denaturation	95 °C	30 sec	30~35 cycles
Annealing/extension	68 °C	1 min/kb	
Final extension	72 °C	Optional. Normally 3~5min	1 cycle

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 5 ul of the reaction mixture directly on agarose gel without adding a loading dye to analyze the PCR products.

Bioneer Corporation
8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302
Republic of Korea
Tel: +82-42-930-8777 (Korea : 1588-9788)
Fax: +82-42-930-8688 E-mail: sales@bioneer.com

Bioneer Inc.
155 Filbert St. Suite 216
Oakland, CA 94607, USA
Toll Free: +1-877-264-4300 Fax: +1-510-865-0350
E-mail: order.usa@bioneer.us.com

Bioneer R&D Center
Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro
Bundang-gu, Seongnam-si, Gyeonggi-do, 13488
Republic of Korea
Tel: +82-31-628-0500 Fax: +82-31-628-0555

IX. Experimental Data

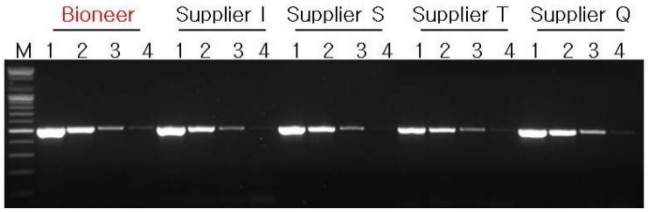

Figure 1. Comparison of PCR amplification between *AccuPower® PyroHotStart Taq* PCR PreMix from Bioneer and other suppliers' Hot start PCR master mix. Sensitivity test was performed by amplifying the IRGC gene from a serial dilution of human genomic DNA. This data shows that *AccuPower® PyroHotStart Taq* PCR PreMix has higher amplification efficiency than other suppliers' Hot start PCR master mix.
Lane M: 100 bp DNA Ladder (Bioneer, Cat. No. D-1030)
Lane 1: 10 ng human genomic DNA
Lane 2: 1 ng human genomic DNA
Lane 3: 100 pg human genomic DNA
Lane 4: 10 pg human genomic DNA

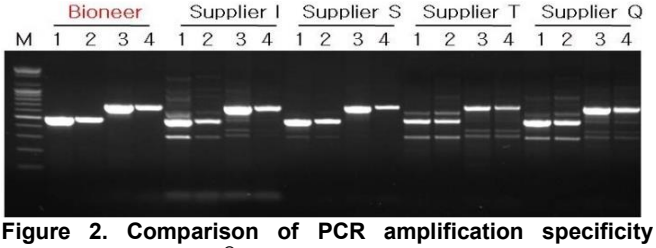

Figure 2. Comparison of PCR amplification specificity between *AccuPower® PyroHotStart Taq* PCR PreMix from Bioneer and other suppliers' Hot start PCR master mix. PCR reactions were performed according to each supplier's protocol. The PrP gene was amplified from human genomic DNA with two different primer sets, separately. This data shows that *AccuPower® PyroHotStart Taq* PCR PreMix has higher amplification efficiency and specificity than other suppliers' Hot start PCR master mix.
Lane M: 100 bp DNA Ladder (Bioneer, Cat. No.D-1030)
Lane 1: 100 ng DNA, PrP primer set (500 bp)
Lane 2: 10 ng DNA, PrP primer set (500 bp)
Lane 3: 100 ng DNA, PrP primer set (705 bp)
Lane 4: 10 ng DNA, PrP primer set (705 bp)

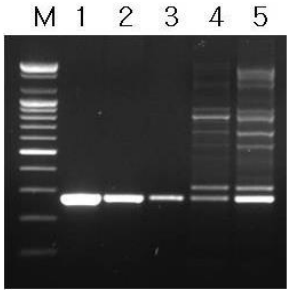

Figure 3. Comparison of PCR amplification specificity between *AccuPower® PyroHotStart Taq* PCR PreMix from Bioneer and other suppliers' Hot start PCR master mix. The ApoE gene was amplifed from 100 ng of human genomic DNA (The PCR product size is 268bp). This data shows that *AccuPower® PyroHotStart Taq* PCR PreMix has higher amplification efficiency and specificity than other suppliers' Hot start PCR master mix.
Lane M: 100 bp DNA Ladder (Bioneer, Cat. No. D-1030)
Lane 1: *AccuPower® PyroHotStart Taq* PCR PreMix
Lane 2: Supplier I *Hotstart Taq* PCR PreMix
Lane 3: Supplier S *Hotstart Taq* PCR master mix
Lane 4: Supplier T *Hotstart Taq* PCR master mix
Lane 5: Supplier Q *Hotstart Taq* PCR master mix

X. Trouble Shooting Guide

- **No product or low yield**

Possible Cause	Recommendation
Insufficient template	Increase the amount of template used in PCR. High quality template is especially essential for amplification of long targets. Check the purity of template or repeat purification of template.
$MgCl_2$ concentration is too low	Increase the amount of $MgCl_2$ concentration in steps.
Primer design is not optimal	Design alternative primers.
Cycle conditions are not optimal	Reduce the annealing temperature. Increase the number of cycles.
Amplification of GC-rich genes	Add 0.5-1 M Betaine or 2-8% DMSO.

- **Product is multi-banded or smeared**

Possible Cause	Recommendation
Annealing temperature is too low	Increase annealing temperature according to primer length.
Incorrect extension time	Adjust the time of the extension step according to the size of the expected PCR product.
Primer design is not optimal	Design alternative primers.
Problems with template	Check the concentration, storage conditions, and quality of template.
Too many cycles	Reduce the number of cycles.

- **Products in negative control experiments**

Possible Cause	Recommendation
Carry-over contamination	Set up PCR reactions in an area separate from that used for PCR product analysis.

XI. Ordering Information

Cat. No.	Description
K-2611	*AccuPower® PyroHotStart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 μl reaction/tube, 96 tubes
K-2612	*AccuPower® PyroHotStart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 μl reaction/tube, 480 tubes
K-2613	*AccuPower® PyroHotStart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached caps, 50 μl reaction/tube, 96 tubes
K-2614	*AccuPower® PyroHotStart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 50 μl reaction/tube, 480 tubes

XII. Notice

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.