BIONEER *AccuPower® GoldHotStart Taq* PCR PreMix

Innovation • Value • Discovery

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I. Introduction

AccuPower® GoldHotstart Taq PCR PreMix is a convenient vacuum-dried PCR master mix containing *GoldHotstart Taq* DNA polymerase, dNTPs, reaction buffer, tracking dye, and patented stabilizer and is aliquoted in 8-strip PCR tubes.

GoldHotstart Taq DNA polymerase is inhibited at lower temperature, but is activated at during the start of PCR. This prevents the formation of misprimed products, as well as primer-dimers, during the reaction set up process resulting in improved specificity.

In addition, *AccuPower® GoldHotstart Taq* PCR PreMix makes hotstart 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
- Highest quality sequencing data

III. Contents

Components	Concentration
GoldHotstart Taq DNA polymerase	1 U
dNTP Mixtures (dATP, dCTP, dGTP, dTTP)	Each 250 μM
Reaction buffer, with 1.5 mM $MgCl_2$	1 X
Stabilizer and tracking dye	

IV. Storage

AccuPower®GoldHotstart Taq PCR PreMix should be stored at -20°C upon receipt and is stable until the expiry date stated on the label

V. Additional Required Materials & Devices

- Thermal cycler for PCR
- Calibrated micropipette
- Sterilized micropipette tips with filters

VI. General Precautions

- Wear gloves throughout 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.

VII. Protocol

1. Thaw template DNA, and primers before use.
2. Add template DNA and primers into the *AccuPower® GoldHotstart Taq* PCR PreMix tubes.
   ♦ Recommended amount of template and primers

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® GoldHotstart Taq* PCR PreMix tubes to a total volume of 20 μl(or 50 μl). Do not calculate the volume of the dried pellet.
4. Dissolve the vacuum-dried Blue pellet completely and spin down 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 30 seconds and then briefly spinning down.
5. Perform the reaction under the following conditions.

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.

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

VIII. Experimental Data

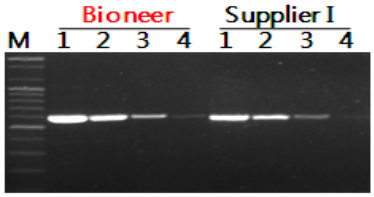

Figure1. Comparison of PCR amplification efficiency between *AccuPower® GoldHotstart Taq* PCR PreMix from Bioneer and other suppliers' Hotstart PCR master mix.

Target gene: GAPDH
Lane M : 100 bp DNA Ladder(Bioneer, Cat. No. D-1030)
Lane 1 : 10 ng of human total cDNA
Lane 2 : 1 ng of human total cDNA
Lane 3 : 100 pg of human total cDNA
Lane 4 : 10 pg of human total cDNA

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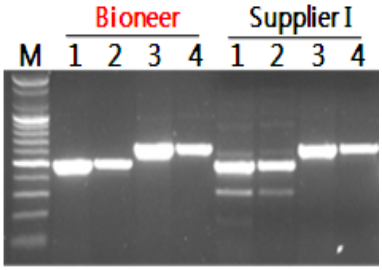

Figure2. Comparison of PCR amplification Specificity between *AccuPower® GoldHotstart Taq* PCR PreMix from Bioneer and other suppliers' Hotstart PCR master mix. The PrP gene was amplified from human genomic DNA with two different primer sets, separately.
Lane M: 100 bp DNA Ladder
Lane 1: 100 ng DNA, PrP set (500 bp)
Lane 2: 10 ng DNA, PrP set (500 bp)
Lane 3: 100 ng DNA, PrP set (705 bp)
Lane 4: 10 ng DNA, PrP set (705 bp)

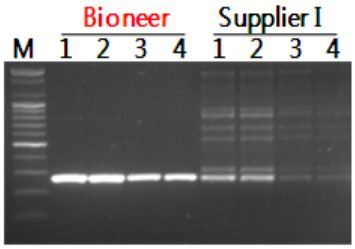

Figure3. Comparison of PCR amplification specificity between *AccuPower® GoldHotstart Taq* PCR PreMix from Bioneer and other suppliers' Hotstart PCR master mix. The ApoE gene was amplifed from 100 ng, 10ng of human genomic DNA.
Lane M: 100 bp DNA Ladder (Bioneer, Cat. No. D-1030)
Lane 1,2: 100 ng human genomic DNA
Lane 3,4: 10 ng human genomic DNA

IX. Trouble Shooting Guide

• Little or no product

Trouble	Recommendation
Insufficient template	Increase the amount of template used in PCR. High quality templates are 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 0.5-1M Betaine or 2-8% DMSO.
Not observed PCR bands	Use an annealing temperature, 5°C lower than the Tm.

• Product is multi-banded or smeared

Trouble	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

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

X. Ordering Information

Cat. No.	Description
K-2621	*AccuPower® GoldHotstart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached caps / 96 tubes, 20 µl reaction/tube
K-2622	*AccuPower® GoldHotstart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached caps / 480 tubes, 20 µl reaction/tube
K-2623	*AccuPower® GoldHotstart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached caps / 96 tubes, 50 µl reaction/tube
K-2624	*AccuPower® GoldHotstart Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached caps / 480 tubes, 50 µl reaction/tube
K-2629	*AccuPower® GoldHotstart* Taq PCR MasterMix, 2.5ml of 2X Master mix solution
K-2630	*AccuPower® GoldHotstart* Taq PCR MasterMix, 25ml of 2X Master mix solution

XI. Notice

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