AccuPower® ProFi Taq PCR PreMix

I. Introduction

AccuPower® ProFi Taq PCR PreMix is a convenient lyophilized PCR master mix containing *ProFi Taq* DNA polymerase, reaction buffer, dNTPs, tracking dye, and a patented stabilizer. *ProFi Taq* DNA polymerase in the premix is a unique recombinant *Taq* DNA polymerase that offers enhanced amplification efficiency and higher fidelity for PCR. *AccuPower® ProFi Taq* PCR PreMix is applicable to any template DNA, and especially effective in amplifying large genomic DNA fragments around 20 kb. *AccuPower® ProFi Taq* PCR PreMix provides accurate long-range amplification of standard and amplification of low-copy target, and is highly suitable for all PCR applications.

II. Application

- Long-range amplification from genomic DNA
- High amplification efficiency
- Excellent performance on difficult templates
- Amplification of low-copy targets
- High yield and high sensitivity PCR

III. Contents

Component \ Reaction Volume	20 μl reaction	50 μl reaction
ProFi Taq DNA polymerase	1 U	2.5 U
dNTPs (dATP, dCTP, dGTP, dTTP)	Each 250 μM	Each 250 μM
Reaction buffer (with 1.5 mM $MgCl_2$)	1 X	1 X
Stabilizer and tracking dye[1)]	Trace	Trace

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

IV. Storage Condition

For long term storage, *AccuPower® ProFi Taq* PCR PreMix should be stored at -20°C and is stable until the expiration 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® ProFi Taq* PCR PreMix tubes.

♦ Reaction mixture example

Component	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
PCR grade water	Variable	Variable

3. Add distilled water into the *AccuPower® ProFi Taq* PCR PreMix tubes to a total volume of 20 μl (K-2631, K-2632) or 50 μl (K-2633, K-2634). Do not calculate the volume of the dried pellet.
4. Dissolve the lyophilized 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 several times and then briefly spinning down.
5. Perform the reaction under the following conditions.

• Standard PCR (3-step)

Step	Temperature	Time	Cycles
Pre-denaturation	95 °C	5 min	1 cycle
Denaturation	95 °C	15-20 sec	25~35 cycles
Annealing	45-65 °C	15~30 sec	
Extension	68 °C	1 min/kb	
Final extension	68 °C	Optional. Normally 3-5 min	1 cycle

Note: Optimal annealing temperature depends on the melting temperature of the primers.

• PCR for long targets longer than 10 kb (2-step)

Step	Temperature	Time	Cycles
Pre-denaturation	95 °C	5 min	1 cycle
Denaturation	95 °C	15~20 sec	30~35 cycles
Annealing/extension	68 °C	1 min/kb	
Final extension	68 °C	Optional. Normally 3~5 min	1 cycle

Note: Annealing/extension time depends on fragment length. Use 15 min for 20 kb, 20 min for 30 kb.

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.

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IX. Experimental Data

Figure 1. Comparison of PCR amplification efficiency between *AccuPower® ProFi Taq* PCR PreMix and other suppliers' PCR master mix.
Target gene: human 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

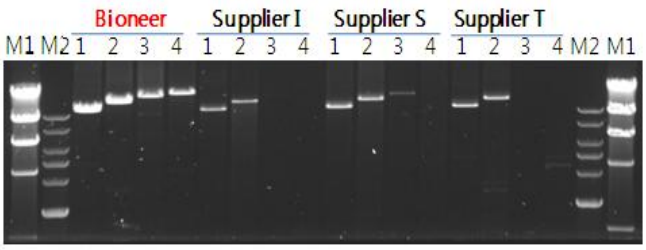

Figure 2. Comparison of PCR amplification of long targets between *AccuPower® ProFi Taq* PCR PreMix and other suppliers' PCR master mix.
Lane M1 : Lambda/Hind III marker (Bioneer, Cat. No. D-1050)
Lane M2 : 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)
Lane 1 : 11 kb fragment
Lane 2 : 13.5 kb fragment
Lane 3 : 17.6 kb fragment
Lane 4 : 21.4 kb fragment

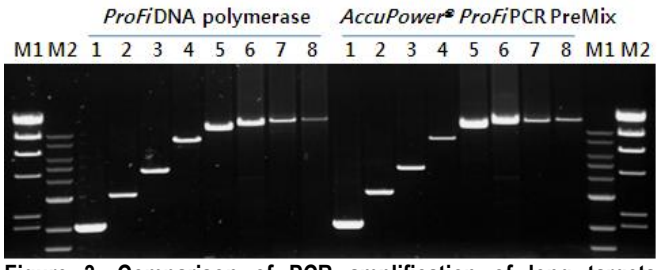

Figure 3. Comparison of PCR amplification of long targets between *ProFi Taq* DNA polymerase and *AccuPower® ProFi Taq* PCR PreMix.
Lane M1 : Lambda/Hind III marker (Bioneer, Cat. No. D-1050)
Lane M2 : 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)
Lane 1 : 2 kb fragment
Lane 2 : 3 kb fragment
Lane 3 : 4.5 kb fragment
Lane 4 : 8 kb fragment
Lane 5 : 11 kb fragment
Lane 6 : 13.5 kb fragment
Lane 7 : 17.6 kb fragment
Lane 8 : 21.4 kb fragment

X. Trouble Shooting Guide

● No product or low yield

Possible Cause	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.

● 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-2631	*AccuPower® ProFi Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 µl reaction/tube, 96 tubes
K-2632	*AccuPower® ProFi Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 µl reaction/tube, 480 tubes
K-2633	*AccuPower® ProFi Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 50 µl reaction/tube, 96 tubes
K-2634	*AccuPower® ProFi Taq* PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 50 µl reaction/tube, 480 tubes