AccuPower® Multiplex PCR PreMix

I. Introduction

AccuPower® Multiplex PCR PreMix is a powerful and simple technology that allows DNA amplification of two or more products in a single tube. The *AccuPower* Multiplex PCR PreMix contains Hotatart *Top* DNA polymerase, dNTPs and reaction buffer in a premixed format that are freeze-dried into an individual packet.

It is widely utilized in genotyping analysis and qualitative and semi-quantitative gene expression analysis using cDNA template.

II. Application

- Genotyping application (e.g., STR or VNTR analysis)
- Semi-quatitative gene expression analysis
- DNA and RNA chip

III. Content

Component	Concentration
Top DNA polymerase	1 U
dNTP(dATP, dCTP, dGTP, dTTP)	Each 250 μM
Reaction Buffer, with 2mM MgCl2	1X
Stabilizer and tracking dye	

IV. Principle

A. PCR (Polymerase Chain Reaction)

PCR is a molecular technique for amplification of target DNA across several orders of magnitude, generating millions or more copies of target DNA fragments. There are three major steps at different temperatures in a PCR, which are repeated for 25 – 45 cycles. Double-stranded target DNA is heat-denatured (denaturation step), the two primers complementary to the target segment are annealed at low temperature (annealing step), and the annealed primers are then extended at an intermediate temperature (extension step) with a DNA polymerase. Theoretically, the target copy number doubles upon each cycle, PCR can thereby amplify DNA fragments up to 10^8-fold in a short period.

B. Pimers

Primer quality is a critical factor for successful multiplex PCR, so *AccuPower* Multiplex PCR PreMix is designed to perform successful multiplex PCR with standard-quality primer pairs. The specificity of all primer pairs should be tested and verified in single PCR reactions before combining them in a multiplex PCR assay.

- Primer design: Primer design is critical to successful multiplex PCR reactions. All primers are designed generally 24 – 35 nucleotides in length and ideally have a Tm value range within 5 °C.
- Annealing temperature: The annealing temperature for multiplex PCR should be chosen using the highest Tm value of the component primer pairs. This decreases non-specific bands in the multiplex PCR reaction.
- Primer molar concentration: The amount of DNA primers available during the PCR reaction influences the results. Too high primer concentrations may inhibit the multiplex reaction whereas too low amounts may not be sufficient. We recommend that final primer concentration of 1 – 5 pmoles per reaction.

C. Agarose gel analysis

Agarose gel electrophoresis is the easiest and most common way of separating and analyzing DNA. The chart below shows recommended agarose concentrations for separating DNA fragments of various sizes.

Efficient range of PCR product	Agarose (%)
200 bp – 2 kb	1.2 – 1.5 %
100 bp – 1 kb	1.5 – 2 %
80 bp – 500 bp	2.5 – 3 %

V. Storage

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

VI. Notice to Purchaser

The *AccuPower* Multiplex PCR PreMix employs an enzyme that is specifically optimized for increasing base incorporation rate by inactivating 5' to 3' exonuclease activity.

VII. Ordering Information

Cat. No.	Description
K-2111	*AccuPower* Multiplex PCR PreMix, 0.2ml thin-wall tubes with attached cap / 96 tubes, 20 μl reaction
K-2112	*AccuPower* Multiplex PCR PreMix, 0.2ml thin-wall tubes with attached cap / 96 tubes, 50 μl reaction
K-2113	*AccuPower* Multiplex PCR PreMix, 0.2ml thin-wall tubes with attached cap / 480 tubes, 20 μl reaction
K-2114	*AccuPower* Multiplex PCR PreMix, 0.2ml thin-wall tubes with attached cap / 480 tubes, 50 μl reaction

VIII. Additionally required materials & Devices

- Thermal cycler for PCR
- Target-specific primers
- Calibrated micropipette
- Sterilized micropipette tips with filters

IX. 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.

AccuPower® Multiplex PCR PreMix

X. Protocol

1. Thaw template DNA, distilled water, and primers before use.
2. Add the template DNA and primers into *AccuPower* Multiplex PCR PreMix tubes.

Components	Amount
Template DNA	1 ng – 100 ng
Primer set	1 – 5 pmoles each

3. Add distilled water into the *AccuPower* Multiplex PCR PreMix tubes to a total volume of 20 µl (K-2111, K-2113) or 50 µl (K-2112, K-2114). Do not calculate the dried pellet.
4. Dissolve the lyophilized green pellet completely and spin down by using Bioneer's *ExiSpin* Vortex/Centrifuge or by pipetting up and down several times and briefly spinning down.
5. Perform the reaction under the following conditions.

Step	Temperature	Time	No. of Cycles
Pre-Denaturation	95 °C	10 min	
Denaturation	95 °C	30 sec	25 – 35 [2]
Annealing	55 – 65 °C [1]	30 – 60 sec	
Extension	72 °C	1 min/kb	
Final Extension	72 °C	5 min	

1) If some bands are missing, lower annealing temperature in 2 – 5°C steps. If non-specific bands exist, increase annealing temperature in 2 – 5°C steps.
2) The number of cycles is dependent on the amount of template DNA.

6. Maintain the reaction mixture at 4 °C after amplification. The sample can 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.

XI. Reaction Example

1. Reaction mixture.

Component	Volume	Concentration
Template	1 µl	100 ng/µl
Primers	2 µl	1 pmole/µl each
D.W	17 µl	
Total	20 µl	

2. Program the PCR setting

Step	Temperature	Time	No. of Cycles
Pre-denaturation	95°C	10 min	1
Denaturation	95°C	30 sec	35
Annealing	57°C / 65°C [1]	30 sec	
Extension	72°C	1 min	
Final-Extension	72°C	5 min	1

1) Annealing temperature : 57°C (a), 65°C (b)

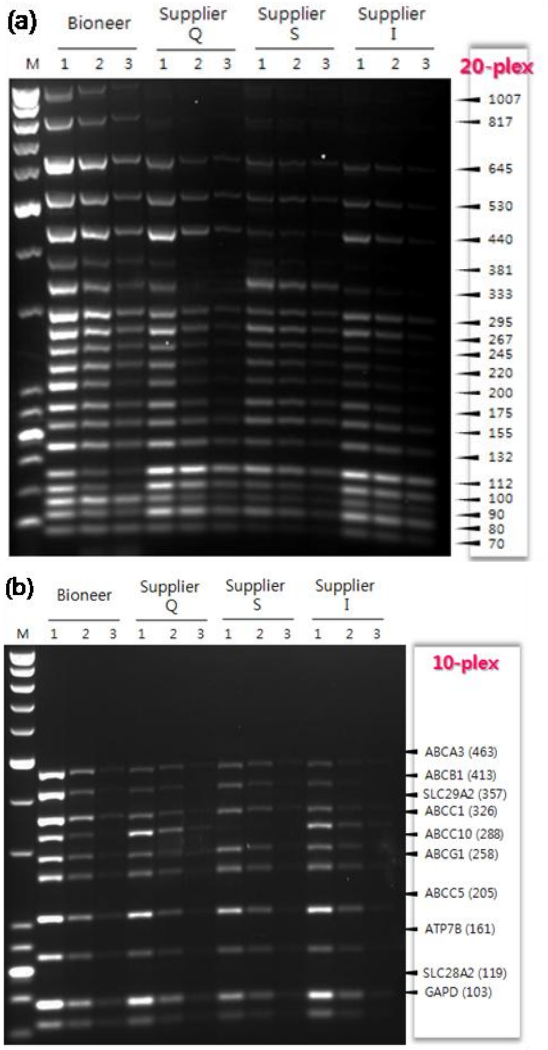

Figure 1. Comparison of amplification quality between *AccuPower* Multiplex PCR PreMix and other supplier's Multiplex PCR kits.
20-plex (a) and 10-plex (b) primer sets were tested using *AccuPower* Multiplex PCR PreMix and other supplier's Multiplex PCR kit. A series of Human genomic DNA diluents were used. (100ng ~ 1ng). All data were obtained using *MyGenie*™ 96 machine (Bioneer co.)
Supplier Q: Q company Multiplex PCR kit, Supplier S: S company Multiplex PCR kit, Supplier I: I company *Taq* polymerase for Mutiplex PCR (0.5U, added 2mM MgCl2)
M; 25/100 bp Mixed DNA Ladder (cat. No. D-1020)