BIONEER
Innovation • Value • Discovery

AccuPower® Gold Multiplex PCR PreMix

Bioneer Corporation	Seoul Office	Bioneer, Inc.	Order
49-3, Munpyeong-dong, Daedeok-gu, Daejeon 306-220 Korea Phone:+82-42-936-8500 Fax: +82-72-930-8600	2nd fl. Samhwa&Sansu B/D, #6 Yangjae-dong, Seocho-ku Seoul, 137-130, Korea Phone:+82-2-598-1094 Fax: +82-2-598-1096	1000 Atlantic Avenue Alameda, CA 94501 USA Toll free : 1-877-264-4300 Fax : 1-510-865-0350 E-mail: order.usa@bioneer.us.com	Korea only: 1588-9788 E-mail: order@bioneer.co.kr URL: www.bioneer.com

I. Introduction

AccuPower® Gold Multiplex PCR PreMix utilizes a powerful and innovative technology that allows DNA amplification of up to 20 products within a single tube. The Multiplex PCR PreMix uses a unique enzyme-mediated HotStart PCR method that provides robust, sensitive, and reliable multiplex PCR results. Bioneer's *Top* DNA Polymerase is completely inhibited by pyrophosphate at temperatures below 70°C. However, *Top* 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 and primer-dimers during the reaction set up process resulting in improved specificity of multiplex PCR.

II. Application

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

III. Contents

Component	Amount
Top DNA polymerase	1 U
Pyrophosphatase and pyrophosphate	
dNTP (dATP, dCTP, dGTP, dTTP)	250 μM each
Reaction Buffer with 2 mM $MgCl_2$	1 x
Stabilizer and tracking dye[1)]	

[1)] *AccuPower* Gold Multiplex PCR PreMix is premixed with Xylene cyanol and Orange G. Xylene cyanol migrates at approximately 4 kb and Orange G migrates at approximately 50 bp.

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. Primers

Primer quality is a critical factor for successful multiplex PCR, so *AccuPower* Gold 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 Gold 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* Gold Multiplex PCR PreMix employs an enzyme that is specifically optimized for increasing base incorporation rate by inactivating 5' to 3' exonuclease activity.

VII. Additional Required Materials & Devices

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

VIII. 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.

IX. Protocol

1. Thaw template DNA, distilled water, and primers before use.
2. Add the template DNA and primers into *AccuPower* Gold 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* Gold Multiplex PCR PreMix tubes to a total volume of 20 μl (K-2115, K-2116) or 50 μl (K-2117, K-2118). 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	5 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.

Bioneer Corporation	Seoul Office	Bioneer, Inc.	Order
49-3, Munpyeong-dong, Daedeok-gu, Daejeon 306-220 Korea Phone:+82-42-936-8500 Fax: +82-72-930-8600	2nd fl. Samhwa&Sansu B/D, #6 Yangjae-dong, Seocho-ku Seoul, 137-130, Korea Phone:+82-2-598-1094 Fax: +82-2-598-1096	1000 Atlantic Avenue Alameda, CA 94501 USA Toll free : 1-877-264-4300 Fax : 1-510-865-0350 E-mail: order.usa@bioneer.us.com	Korea only: 1588-9788 E-mail: order@bioneer.co.kr URL: www.bioneer.com

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.

X. 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. PCR cycling condition

Step	Temperature	Time	No. of Cycles
Pre-denaturation	95°C	5 min	1
Denaturation	95°C	30 sec	30
Annealing	57°C[1)]/65°C[2)]	30 sec	
Extension	72°C	1 min	
Final-Extension	72°C	5 min	1

[1)] Figure 1, Figure 2. (a)
[2)] Figure 2. (b)

XI. Experimental Data

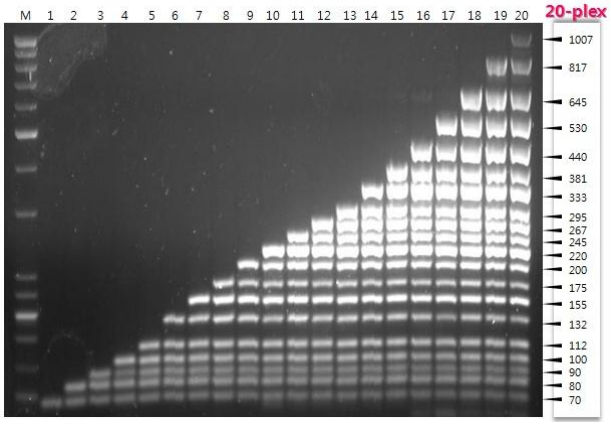

Figure 1. High specificity of *AccuPower* Gold Multiplex PCR PreMix. Each line from left to right represents the progressive number of primer sets up to 20 included in *AccuPower* Gold Multiplex PCR PreMix.

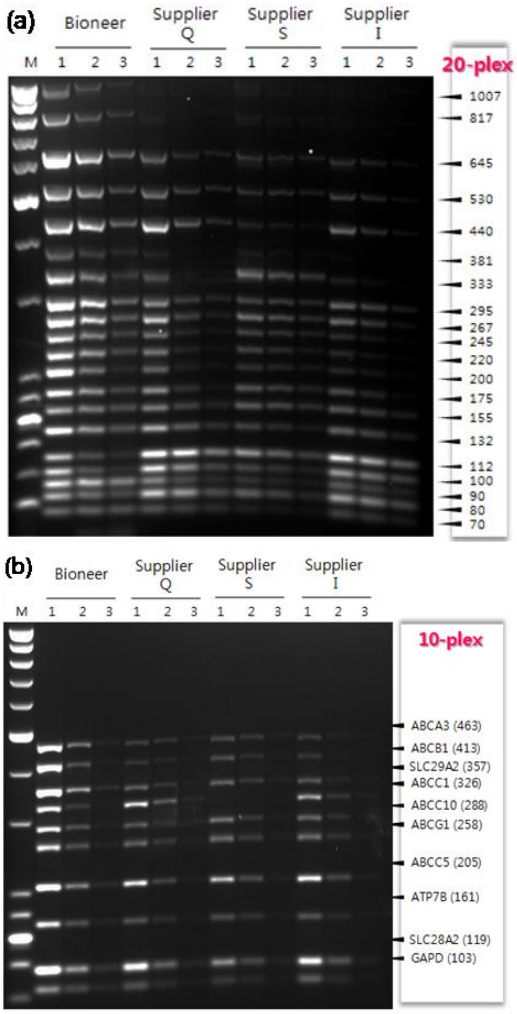

Figure 2. Comparison of amplification quality between *AccuPower* Gold Multiplex PCR PreMix and other supplier's Multiplex PCR kits.

XII. Ordering Information

Cat. No.	Description
K-2115	*AccuPower* Gold Multiplex PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 µl reaction, 96 tubes
K-2116	*AccuPower* Gold Multiplex PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 µl reaction, 480 tubes
K-2117	*AccuPower* Gold Multiplex PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 50 µl reaction, 96 tubes
K-2118	*AccuPower* Gold Multiplex PCR PreMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 50 µl reaction, 480 tubes