AccuPower® Plus *DualStar*™ qPCR PreMix [Plus *DualStar*™ qPCR Master Mix(2x)]

(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® Plus *DualStar*™ qPCR PreMix is a ready-to-use reagent containing all components necessary for real-time PCR, except for template, target-specific primers and fluorogenic probe. The kit is designed to provide reproducible results with high sensitivity and specificity, even in the presence of PCR inhibitors. *AccuPower*® Plus *DualStar*™ qPCR PreMix exhibits a wide dynamic range providing reliable amplification over 7 orders of magnitude. Sensitivity and specificity are ensured by the use of Bioneer's Hotstart *Taq* DNA polymerase.

This product can be used for hydrolysis probe-based real-time PCR experiments for the amplification and detection of genomic DNA and cDNA targets, differential gene expression profiling, SNP (Single Nucleotide Polymorphism) analysis, and evaluation of RNAi products. This product provides reproducible results with superior specificity, high sensitivity, wide dynamic range and accurate quantification.

II. Principle

PCR products are detected with TaqMan® probe in real-time monitoring.

1) PCR (Polymerase Chain Reaction)

PCR is a biochemistry and molecular biology technique for amplification of target DNA across several orders of magnitudes, generating millions or more copies of target DNA pieces.

There are three major steps at different temperatures in a PCR, which are repeated for 30 or 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.

2) Fluorescence detection

TaqMan assays, also referred to as 5'-nuclease assays, use the 5' to 3' exonuclease activity of *Taq* DNA polymerase. Each reaction contains a gene specific primer and a fluorescence dye labeled TaqMan probe. The probe contains a 5' reporter dye (e.g. FAM) and a 3' quencher dye (e.g. TAMRA). The 3'-end is also blocked to prevent extension during PCR. The probe is designed to anneal the target sequence between the forward and reverse PCR primers. While the probe is intact, the quencher suppresses the fluorescence of the reporter dye. During amplification, *Taq* DNA polymerase cleaves the probe and displaces it from the target, allowing extension to continue. Cleavage of the probe separates the reporter dye from the quencher dye, resulting in an increase of fluorescent intensity. The increased fluorescence only occurs if the target sequence is amplified and is complimentary to the probe, thus preventing detection of non-specific amplification. For any given cycle within the exponential phase, the amount of product, and hence fluorescence signal, is directly proportional to the initial copy number. Thus, Ct (threshold cycle) of higher copy number templates will be lower compared to that of lower copy templates.

III. Content

Cat. No	Size	Descriptions
K-6600	96 tests	*AccuPower* Plus *DualStar* qPCR PreMix, *Exicycler*™ 96, 12 strips, *Exicycler* 8-well strip, 50 ㎕/rxn, optical film included
K-6601	96 tests	*AccuPower* Plus *DualStar* qPCR PreMix, ABI7500, 12 strips, ABI7500 8-well strip, 50 ㎕/rxn,, optical film included
K-6602	96 tests	*AccuPower* Plus *DualStar* qPCR PreMix, Opticon, 12 strips, Opticon 8-well strip, 50 ㎕/rxn,, optical film included
K-6603	100 tests	*AccuPower* Plus *DualStar* qPCR Master Mix (2X)

Cat. No	Kit Contents
K-6600	8-well strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes
K-6601	8-well strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes * ROX dye (50X) 0.1 ml x 1 tube
K-6602	8-well strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes
K-6603	2X Master Mix 0.625ml x 4 tubes DEPC-D.W. 1.2 ml x 1 tubes * ROX dye (50X) 0.1 ml x 1 tube

* ROX dye is used for normalization of intensity by background subtraction. The use of ROX dye is recommended for Applied Biosystems 7500 Real-Time PCR System.

* The use of ROX dye is not required for Bioneer *Exicycler* 96 Real-Time PCR System.

IV. Storage

For long term storage, Plus *DualStar* qPCR PreMix should be stored at -20°C upon receipt and is stable until the expiration printed on the label.

V. Additionally Required Materials & Devices

- Thermal Cycler for real-time PCR (authorized instruments)
- Target-specific primers and TaqMan-based probe
- Calibrated micropipette
- Sterilized micropipette tips with filters
- Optical adhesive films for real-time PCR
- High-speed centrifuge with rotors for microtiter pates
- Vortex mixer, Desktop centrifuge
- Disposable powder-free gloves

VI. General precautions

- Wear gloves during experiments to prevent contaminatio n.
- Store positive materials, such as samples and control t emplates, in a separate freezer from the kit.
- Add templates to the reaction mixture in a hood or a s patially separated facility.

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

VII. Protocol

1.Add following PCR reagents into your PCR tube(s) or Plate.

PreMix (K-6600, K-6601, K-6602)	
Components	**Final Concentration**
PCR Forward-Primer	5 to 50 pmole
PCR Reverse-Primer	5 to 50 pmole
TaqMan® Probe	5 to 50 pmole
Template	10 pg to 100 ng
(Optional) 50 X ROX dye	1 X
DEPC-distilled water.	Adjust to final volume
2X Master Mix (K-6603)	
Components	**Final Concentration**
2X Master Mix	1 X
PCR Forward-Primer	5 to 50 pmole
PCR Reverse-Primer	5 to 50 pmole
TaqMan® Probe	5 to 50 pmole
Template	10 pg to 100 ng
(Optional) 50 X ROX dye	1 X
DEPC-distilled water.	Adjust to final volume

2.Seal the tubes or plate using Optical adhesive film for real-ti me PCR or optically clear cap strips.

3.Completely mix by vortexing (or by pipetting up and down s everal times before sealing the reactions).

4.Centrifuge at 3,000 rpm, for 2 min (optional – necessary onl y if mixing was performed by vortexing)

5.Load the tube or plate onto your Real-time PCR instrument.

6.Program PCR settings as follows:

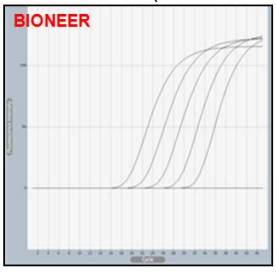
Step	Condition	Cycle
Pre-Denaturation	95 °C, 3-5 min	1
Denaturation	95 °C, 5-30 sec	40-45
Annealing/Extension /Detection	55-60 °C, 30-35 sec	

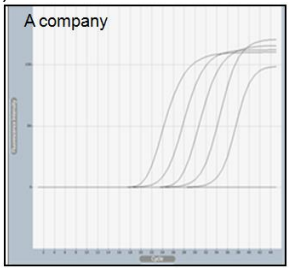
7.After reaction is completed, perform data analysis.

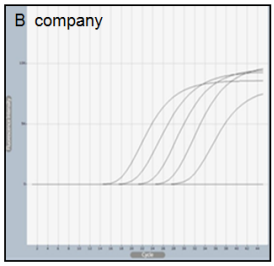
**** This recommended protocol can be modified to optimize results, based on the real-time PCR instrument and target DNA sequences***.

VIII. Experimental Example

Figure 1. Data using *AccuPower* Plus *DualStar* qPCR PreMix
Comparison of amplification quality between *AccuPower* Plus *DualStar* qPCR PreMix and other supplier's Real time qPCR kit. All data were obtained using *Exicycler* 96 Real-time Quantitative Thermal Block (Bioneer Co.).





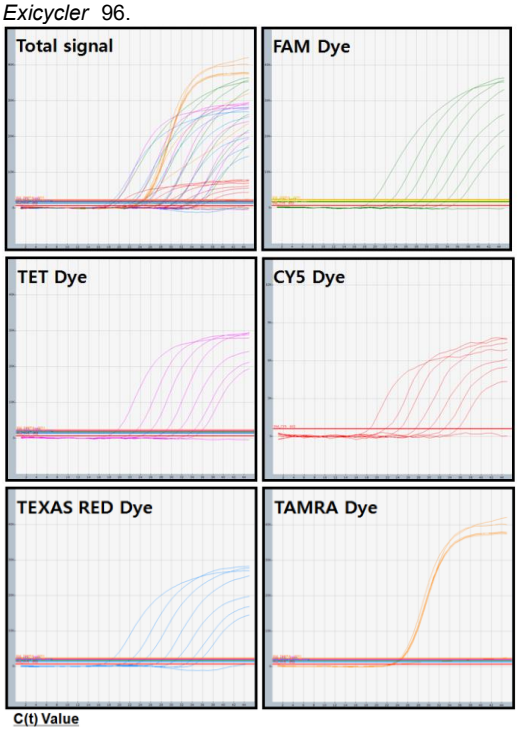


C(t) Value

Template DNA copies	BIONEER	A Company	B Company
1000	32.6	34.72	31.28
10000	29.18	31.31	27.75
100000	25.66	27.61	24.44
1000000	22.43	24.48	21.04
10000000	19.22	20.98	17.99
Efficiency /linearity	99% / 1	96% / 1	100% / 0.9999

Five-target multiplexing on the *Exicycler* 96 instrument using *AccuPower* Plus *DualStar* qPCR

The figure shows amplification of a 5-target multiplex assay. The dyes used were FAM, TET, CY5, Texas Red and TAMRA (internal positive control), respectively. The data demonstrate that over a dilution series of input template, the *AccuPower* Plus *DualStar* qPCR can successfully and reliably generate up to 5-target multiplex data on the *Exicycler* 96.



C(t) Value

Dye	Template DNA of copies							
	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02	1.00E+01	NTC
FAM	19.13	22.8	25.17	28.48	31.53	33.95	35.98	UD
TET	19.27	23.11	25.83	28.89	31.31	34.15	35.76	UD
CY5	18.48	21.52	24.33	27.35	30.02	32.86	35.45	UD
TEXAS_RED	18.19	21.56	24.21	27.8	30.42	33.39	35.19	UD
	1.00E+05	1.00E+05	1.00E+05	1.00E+05	1.00E+05	1.00E+05	1.00E+05	NTC
TAMRA (IPC)	24.65	24.75	24.51	24.41	24.66	24.73	24.83	UD

IX. Notice

Bioneer corporation reserves 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.