AccuPower® Plus *DualStar*™ qPCR PreMix, MasterMix (2X)

I. Introduction

AccuPower® Plus *DualStar*™ qPCR PreMix is a ready-to-use reagent containing all components for real-time PCR reaction, except for target-specific primers and fluorogenic probe. Just addition of primers and probe specific to gene of interest into tube provide reproducible results with high sensitivity and specificity. Because all components for PCR reaction are lyophilized in real-time PCR plates or tubes, the stability of product can be extremely extended up to 2 years at -20 °C storage, compared to that of other commercially available product.

This product can be used in 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 the reproducible results with the 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. As the target copy number doubles upon each cycle, PCR can thereby amplify DNA fragments up to 10^8-fold in a short period.

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 μℓ/rxn, optical film included
K-6601	96 tests	*AccuPower* Plus *DualStar* qPCR PreMix, ABI7500, 12 strips, ABI7500 8-well strip, 50μℓ/rxn, optical film included
K-6602	96 tests	*AccuPower* Plus *DualStar* qPCR PreMix, Opticon, 12 strips, Opticon 8-well strip, 50μℓ/rxn, optical film included
K-6603	100 tests	*AccuPower* Plus *DualStar* qPCR Master Mix (2X)

Cat. No	Kit Contents
K-6600	96-well plate x 1 each DEPC-D.W. 1.2 ml x 4 tubes
K-6601	96-well plate x 1 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 (50X) is recommended for Applied Biosystems 7500 Real-Time PCR System.

* The use of ROX dye is not required for Bioneer *Exicycler* 96 and Bio-Rad DNA engine Opticon, iCycler IQ5 real-time instruments.

IV. Storage

For long term storage, Plus *DualStar* qPCR PreMix should be stored at -20°C upon receipt and is stable until the expiry date stated 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 plates
- Vortex mixer
- Desktop centrifuge
- Disposable powder-free gloves

VI. General precautions

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

VII. Protocol

1. Add following PCR reagents into Plus *DualStar* qPCR PreMix tube (per reaction)

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

2. Seal the Optical adhesive film for real-time PCR on tube or plate.
3. Completely mix by vigorous vortexing for resuspension of PreMix pellets.
4. Centrifuge at 3,000 rpm, for 2 min.
5. Start Real-time PCR instrument and load it.
6. Program the PCR setting.

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.

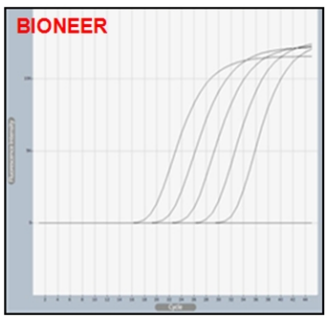
**** This recommended protocol can be modified to get the optimal results, based on used real-time PCR instrument and target DNA sequences***.

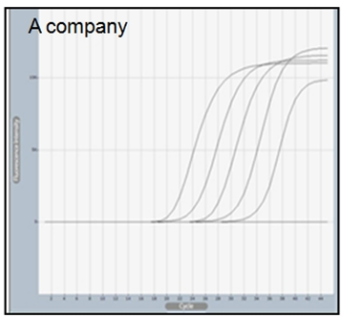
VIII. Experimental Example

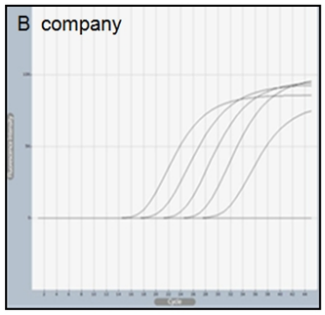
Figure 1. Data using *AccuPower* Plus *DualStar* qPCR PreMix

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.).





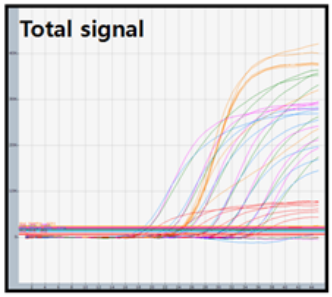


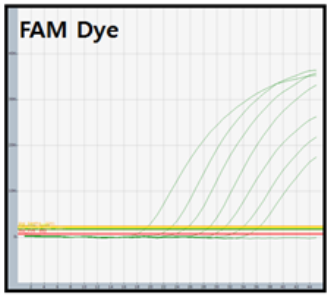
C(t) Value

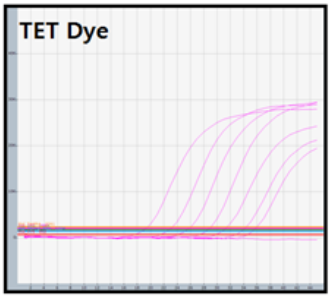
Template DNA copies	BIONEER	A Company	B Company
1000	32.6	34.72	31.28
10000	29.18	31.31	27.75
100000	25.66	27.61	24.44
1000000	22.43	24.48	21.04
10000000	19.22	20.98	17.99
Efficiency /linearity	99% / 1	96% / 1	100% / 0.9999

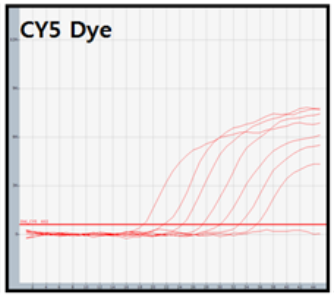
Figure 2. Five-target multiplexing on the *Exicycler* 96 instrument using *AccuPower* Plus *DualStar* qPCR PreMix

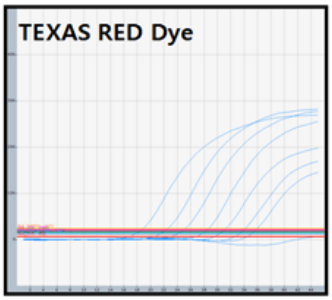
Figure 2 shows amplification of a 5-target multiplex assay. The dyes used were FAM, TET, CY5, Texas Red and TAMRA, respectively. The data demonstrate that over a dilution series of input template, the *AccuPower* Plus *DualStar* qPCR PreMix can successfully and reliably generate up to 5-target multiplex data on the *Exicycler* 96.

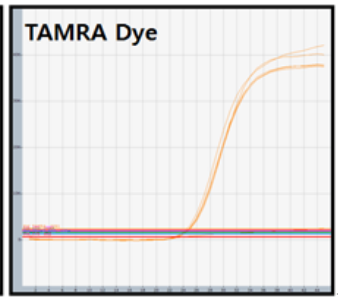












C(t) Value

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