AccuPower® DualStar™ qPCR PreMix

I. Introduction

AccuPower® 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-6100	96 tests	*Exicycler™* 96, 8-well strip, 20 μℓ reaction
K-6110	96 tests	*Exicycler™* 96, 8-well strip, 50 μℓ reaction
K-6101	96 tests	ABI 7500, 8-well strip, 20 μℓ reaction
K-6111	96 tests	ABI 7500, 8-well strip, 50 μℓ reaction
K-6102	96 tests	Opticon®, 8-well strip, 20 μℓ reaction
K-6112	96 tests	Opticon®, 8-well strip, 50 μℓ reaction
K-6103	96 tests	*Exicycler™* 96,96-well plate, 20 μℓ reaction
K-6113	96 tests	*Exicycler™* 96,96-well plate, 50 μℓ reaction
K-6104	96 tests	ABI 7500,96-well plate, 20 μℓ reaction
K-6114	96 tests	ABI 7500,96-well plate, 50 μℓ reaction

Cat. No	Kit Contents
K-6100 K-6101 K-6102	8-well strip x 12 each DEPC-D.W. 1.2 ml x 2 tubes * ROX dye (50X) 0.2 ml x 1 tube (only in K-6101)
K-6110 K-6111 K-6112	8-well strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes * ROX dye (50X) 0.2 ml x 1 tube (only in K-6111)
K-6103 K-6104	96-well plate x 1 each DEPC-D.W. 1.2 ml x 2 tubes * ROX dye (50X) 0.2 ml x 1 tube (only in K-6104)
K-6113 K-6114	96-well plate x 1 each DEPC-D.W. 1.2 ml x 4 tubes * ROX dye (50X) 0.2 ml x 1 tube (only in K-6114)

* 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

AccuPower DualStar qPCR PreMix should be stored at – 20℃ upon received, and are 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 separated freezer from freezers for the kit.

- Add templates to the reaction mixture in clean bench or a spatially separated facility
- Place tubes / plate at room temperature at least 5 min before use.
- **Vortex and centrifuge briefly tubes before load tubes into instruments.**

VII. Protocol

Recommended Protocol Using *Exicycler* 96 version 3.0 (Bioneer Co.), Applied Biosystems 7500 Real-time PCR System (Applied Biosystems) and DNA Engine Opticon (Formerly, MJ Research; Bio-Rad Inc.)

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

	20 μℓ Rxn	50 μℓ Rxn
PCR F-Primer (10 pmole)	1-2 μℓ	2-5 μℓ
PCR R-Primer (10 pmole)	1-2 μℓ	2-5 μℓ
TaqMan® Probe	1-2 μℓ	2-5 μℓ
Template	5 μℓ	10 μℓ
DEPC-distilled water.	Adjust to 20 μℓ	Adjust to 50 μℓ

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

1. Target : Envelope gene of West Nile Virus (WNV)
2. Primer/Probe: designed using Primer3 Plus & purchase from Bioneer Co. (SOUTH KOREA)
3. Template: plasmid DNA containing Envelop gene region of WNV
4. Used Reagent composition (per 20 μℓ reaction)

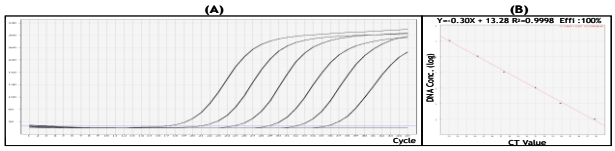
WNV Forward Primer (10 pmole)	1 μℓ
WNV Reverse Primer (10 pmole)	1 μℓ
WNV TaqMan Probe(10 pmole)	1 μℓ
Template(10^7 ~ 10^2 copies/rxn)	5 μℓ
DEPC-distilled water.	12 μℓ
Final	20 μℓ

5. Program the PCR setting

Step	Condition	Cycle
Pre-Denaturation	95 °C, 5 min	1
Denaturation	95 °C, 20 sec	45
Annealing/Extension /Detection (Scan)	55 °C, 30 sec	

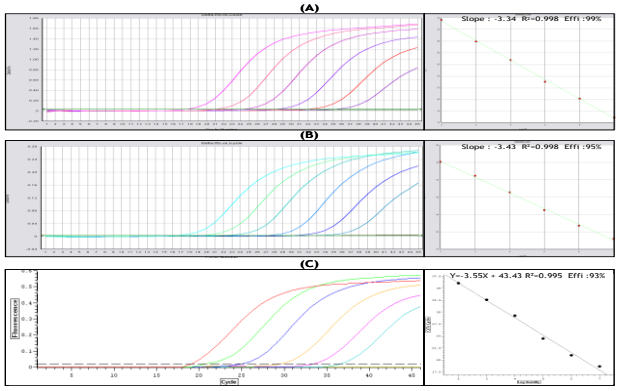
6. Perform Data analysis

Figure 1. Data using *AccuPower DualStar* qPCR PreMix



AccuPower DualStar qPCR PreMix provides at least 6 orders of magnitude in dynamic range (10^7 ~ 10^2 copies/reaction).
(A) Amplification curve, (B) Standard curve. All data were obtained using *Exicycler* 96 Real-time Quantitative Thermal Block (Bioneer Co.).

Figure 2. Data using various kinds of Real-time PCR Instruments



AccuPower DualStar qPCR PreMix is applicable to most of commercially available real-time quantitative PCR instruments. West Nile Virus (WNV) primers and TaqMan-based probe were added into *DualStar* qPCR PreMix. A series of WNV positive control diluents were tested.
(A) Amplification curve and standard curve using ABI 7500 Fast Real-time PCR machine (Applied Biosystems).
(B) Amplification curve and standard curve using ABI 7500 Real-time PCR machine (Applied Biosystems).
(C) Amplification curve and standard curve using DNA Engine Opticon Real-time PCR machine (MJ Research, currently Bio-Rad Inc.).

IX. . Related Products

Cat. No.	Product
K-6200~ K-6202	*AccuPower GreenStar*™ qPCR PreMix, *Exicycler* ™ 96, ABI 7500, Opticon 8-well strip, 96 tests /pkg
K-6203~ K-6204	*AccuPower GreenStar*™ qPCR PreMix,*Exicycler* ™ 96, ABI 7500 96-well plate, 96 tests /pkg
K-3032	*AccuPrep*™ Genomic DNA Extraction Kit, 100 extractions
K-3033	*AccuPrep*™ Viral RNA Extraction Kit, 100 extractions
A-2040	*Exicycler*™ 96 Real-time Quantitative Thermal Block