AccuPower® Plus *DualStar*™ qPCR PreMix (With UDG) [2X Plus *DualStar* ™ qPCR Master Mix (With UDG)] (V2/2018-04-12)

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I. Introduction

AccuPower® Plus *DualStar*™ qPCR PreMix / 2X Master Mix (with UDG; Uracil DNA Glycosylase) is a ready-to-use reagent containing all components necessary for real-time PCR, with exception of template, target-specific primers and fluorogenic probe. This kit is designed to provide reproducible results with high sensitivity and specificity, even in the presence of PCR inhibitors, as well as to prevent carryover contamination through UDG. It exhibits a wide dynamic range of over 8 logs of magnitude, which provides reliable amplification. Sensitivity and specificity are also 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.

II. Principle

PCR products are detected with TaqMan® probe in real-time monitoring with enhanced sensitivity through UDG-related removal of carryover contamination.

1) PCR (Polymerase Chain Reaction)

PCR is a biochemical and molecular biological 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 the PCR, which are repeated for 30 to 45 cycles generally. 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 the 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. The cleavage of the probe separates the reporter dye from the quencher dye, resulting in an increase of fluorescent intensity. The increase of fluorescence only occurs if the target sequence is amplified and is complimentary to the probe, thus it prevents the 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, the Ct (threshold cycle) of higher copy number templates will be lower compared to that of lower copy number of templates.

3) UDG (Uracil DNA Glycosylase)

AccuPower® Plus *DualStar*™ qPCR PreMix / 2X Master Mix (with UDG) contains uracil DNA glycosylase, dA, dG, dC with dUTP and reaction buffer in the premixed format that is vacuum dried into individual tubes. The carryover contamination is a significant source of error when PCR is being used in a diagnostic context. The use of uracil DNA glycosylase, which catalyzes the hydrolysis of N-glycosylic bond between the uracil and sugar, is a solution for this problem (Figure 1). UDG efficiently removes the uracil from single-stranded or double-stranded DNA. Incubating of uracil-contained DNA from previous PCR with dUTP at 37°C leads degradation of the DNA, through activation of UDG, while original template DNA remains intact. Therefore, it is possible to do a second PCR with the original template DNA without intervention of previous PCR products.

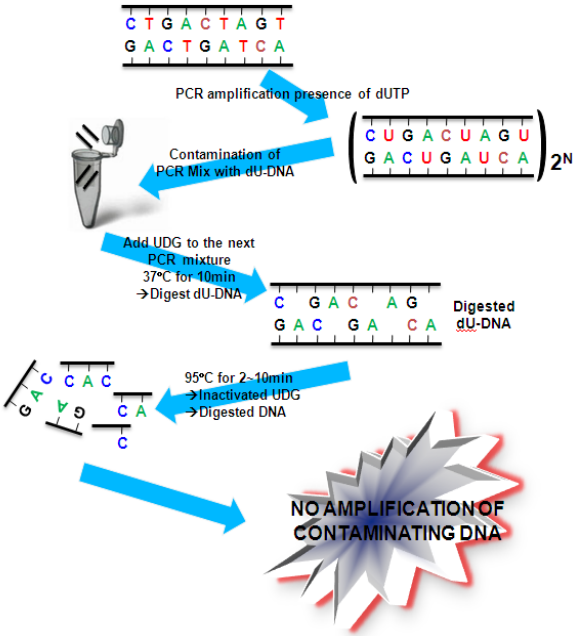

Figure 1. Prevention of carryover contamination.

III. Description and Contents of the kit

Cat. No	Size	Description
K-6605	96 tests	*AccuPower*® Plus *DualStar*™ qPCR PreMix (with UDG), *Exicycler*™ 96, 12 strips, *Exicycler*™ 8-tube strip, 50 µl/rxn, optical film included
K-6606	96 tests	*AccuPower*® Plus *DualStar*™ qPCR PreMix (with UDG), ABI7500, 12 strips, ABI7500 8-tube strip, 50 µl/rxn, optical film included
K-6607	96 tests	*AccuPower*® Plus *DualStar*™ qPCR PreMix (with UDG), Opticon, 12 strips, Opticon 8-tube strip, 50 µl/rxn, optical film included
K-6608	100 tests	*AccuPower*® Plus *DualStar*™ qPCR 2X Master Mix (with UDG)

Cat. No	Kit Contents
K-6605	8-tube strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes
K-6606	8-tube strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes * ROX dye (50X) 0.1 ml x 1 tube
K-6607	8-tube strip x 12 each DEPC-D.W. 1.2 ml x 4 tubes
K-6608	2X Master Mix 0.625 ml x 4 tubes DEPC-D.W. 1.2 ml x 1 tube * ROX dye (50X) 0.1 ml x 1 tube

* ROX dye is used for normalization of intensity by background subtraction.

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IV. Storage

For long-term storage, *AccuPower*® Plus *DualStar*™ qPCR 2X Master Mix (with UDG) 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
- 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 and Desktop centrifuge
- Disposable powder-free gloves

VI. Protocol

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

PreMix (K-6605, K-6606, K-6607)	
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 of 50 µl
2X Master Mix (K-6608)	
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 of 50 µl

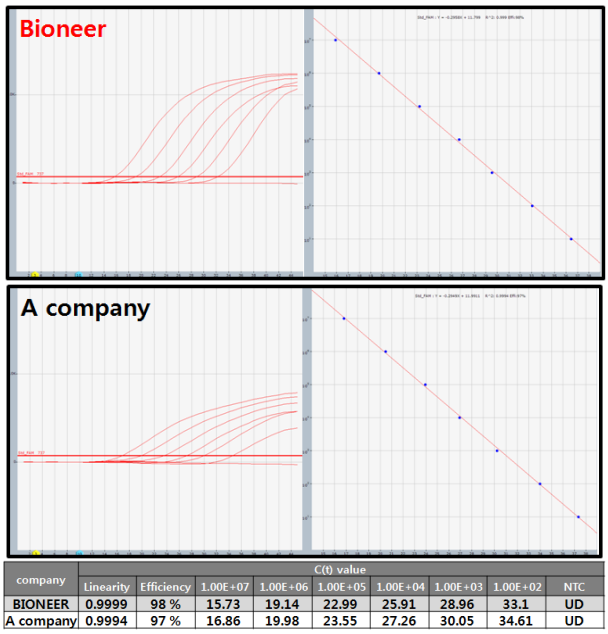
2. Seal the tubes or plate using Optical adhesive film for real-time PCR or optically clear cap strips.
3. Completely mix by vortexing (or by pipetting up and down several times before sealing the reactions).
4. Centrifuge at 3,000 rpm, for 2 min (optional – necessary only if
   mixing was performed by vortexing)
5. Load the tube or plate onto your Real-time PCR instrument.
6. Program PCR settings as follows

Step	Condition	Cycle
UDG Activation	37°C, 2 min	1
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.

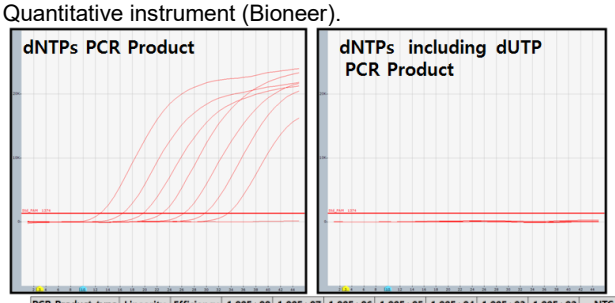
**** This recommended protocol can be modified for further optimization, based on the Real-time PCR instrument and/or target DNA sequences.***

VII. Experimental Example



company	C(t) value								
	Linearity	Efficiency	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02	NTC
BIONEER	0.9999	98 %	15.73	19.14	22.99	25.91	28.96	33.1	UD
A company	0.9994	97 %	16.86	19.98	23.55	27.26	30.05	34.61	UD

Figure 1. Comparison of real-time PCR amplification using *AccuPower*® Plus *DualStar*™ qPCR PreMix / 2X Master Mix (with UDG) (Bioneer) and other company's Real-time qPCR kit. All data were obtained using *Exicycler*™ 96 Real-time Quantitative instrument (Bioneer).



PCR Product type	Linearity	Efficiency	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02	NTC
dNTPs	0.9988	98	12.81	16.41	19.72	22.84	26.37	29.76	33.48	UD
dNTPs including dUTP	-	-	UD	UD	UD	UD	UD	UD	UD	UD

Figure 2. Efficiency of uracil DNA glycosylase using PCR products (including dNTP/dUTP base).
Real-time qPCR experiments were performed with *AccuPower*® Plus *DualStar*™ qPCR PreMix (with UDG) using either dNTPs or dUTP-contained dNTPs. Note that UDG cleaves the DNA containing uracil, so that it suppresses amplification of carryover products.

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VIII. 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.

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