AccuPower® 2X GreenStar™ qPCR Master Mix

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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® 2X *GreenStar*™ qPCR MasterMix is a ready-to-use reagent containing all components for real-time PCR reaction, except for target-specific primers.

This product can be used in real-time PCR experiments for the amplification and detection of genomic DNA and cDNA targets, differential gene expression profiling, and Microbial & Viral pathogen detection. This product provides reproducible results with superior specificity, high sensitivity, wide dynamic range and accurate quantification.

II. Components.

The *AccuPower*® 2X *GreenStar*™ qPCR MasterMix is supplied in a convenient 2X concentration Master Mix to perform real-time PCR. It contains;

- dsDNA-binding fluorescent dye
- Hotstart DNA polymerase (1 U)
- dNTP Mixture (each 250 uM)
- Optimized buffer components (contains Tris-HCl, 60 mM KCl,1.5 mM $MgCl_2$)
- Stabilizer

III. Content

Cat. No.	Size	Kit Contents
K-6251	100rxn (50 μl reaction)	2X Master Mix 0.625 ml x 4 tubes DEPC-D.W. 1.2 ml x 1 tubes * ROX dye (80X) 0.6 ml x 1 tube
K-6252	200rxn (50 μl reaction)	2X Master Mix 0.625 ml x 8 tubes DEPC-D.W. 1.2 ml x 1 tubes * ROX dye (80X) 0.6 ml x 2 tube
K-6253	100rxn (50 μl reaction)	2X Master Mix 0.625 ml x 4 tubes DEPC-D.W. 1.2 ml x 1 tubes
K-6254	200rxn (50 μl reaction)	2X Master Mix 0.625 ml x 8 tubes DEPC-D.W. 1.2 ml x 1 tubes

IV. Storage Condition

AccuPower® 2X *GreenStar*™ qPCR MasterMix should be stored at -20°C and is stable until the expiration date stated on the label.

V. Compatible Instruments

- Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System.
- StepOnePlus Real-Time PCR System (Life Technologies)
- LightCycler/LightCycler 480 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad)

VI. Protocol

1. Thaw template DNA, and primers before use.
2. Add *AccuPower*® 2X *GreenStar*™ qPCR MasterMix, template DNA and primers into the real-time PCR tubes.
   • Recommended amount of template and primers.

Component	20 μl Reaction	50 μl Reaction
2X GreenStar Master Mix	10 μl	25 μl
Template DNA	5 pg~100 ng	5 pg~100 ng
Forward primer (10 pmol/μl)	0.5~2 μl	1~5 μl
Reverse primer (10 pmol/μl)	0.5~2 μl	1~5 μl
(Optional) Rox Dye[1)]	0.25~2 μl	0.625~5 μl
PCR grade water	variable	variable

*1) 80X ROX reference dye must be added when using real-time cyclers that require a passive reference dye.

- Recommended ROX dye concentration.

Real-time cycler	Dye concentration
Exicycler,Roche cyclers, Bio-Rad cyclers etc.	Not required
Applied Biosystems 7900HT etc.	8X
Applied Biosystems Step one, Step one plus	4X
Applied Biosystems 7500, 7500Fast	1X

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

• PCR condition (2 step)

Step	Temperature	Time	Cycles
Pre-denaturation	95°C	1~15 min[2)]	1 cycle
Denaturation	95°C	3~15 sec[3)]	40~45 Cycles
Annealing/extension	60°C	5~30 sec	
Melting			1 step

*2)-1The recommended pre-denaturation time on each real-time cyclers

Real-Time cycler	Pre-denaturation time
General real-time cyclers (Exicycler,ABI7500,7900HT,CFX)	5 min
High speed cycler(e.g. ABI 7500Fast)	1 min

*2)-2 Initial activation time for chemical hotstart *Taq* polymerase is 15 min.

*3) The recommended denaturation time on each real-time cyclers

Real-Time cycler	Denaturation time
General real-time cyclers (Exicycler,ABI7500,7900HT,CFX)	15 sec
High speed cycler(e.g. ABI 7500Fast)	3 sec

• PCR condition (3 step)

For 3-step cycling protocols, Annealing temperature should be set at primer's Tm -5°C for 5~30 sec. Optimal extension temperature is 72°C for 30 sec.

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VII. Primer Design

The primer should be designed according to the following suggestions;

- Primer length: 17~25 mer
- GC content of primer: 40-60%
- Target length: ≤ 200 bp (optimally, 80-150 bp)
- Recommend Tm: between 58–65°C
- Avoid any GC-rich or AT-rich sequence at the 3' end.

How to design primer for *Accupower*?

- http://carbon.bioneer.co.kr/primer3plus/

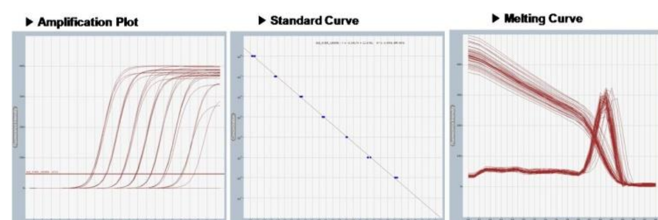
VIII. Template DNA

- cDNA
  Non-purified cDNA, generated by reverse transcription reactions, can be used directly for real-time PCR (Up to 10%).
- Genomic DNA, Viral RNA
  Genomic DNA and viral RNA can be used at up to 100 ng in 50 µl reactions.
- Plasmid DNA
  Although super-coiled plasmids can be used, linearized plasmid DNA produces more accurate assays.

IX. Experimental Example

Figure 1. Highly reproducible Ct values

Amplification of an 90-bp target DNA sequence was detected using serially diluted LP(Legionella Pneumoniae) genomic DNA (from 10^6 copies to 10 copies) with AccuPower 2X Greenstar qPCR Master mix. As shown in Fig. 1, highly reproducible Ct values were achieved within each Lot. set of triplicates



	Batch 1	Batch 2	Batch 3	Batch 4
Efficiency	96	97	94	95
linearity	0.9986	0.9993	0.9993	0.9996
LP 10^7	15.56	15.61	15.21	15.31
LP 10^6	18.71	18.75	18.53	18.72
LP 10^5	22.37	22.28	22.25	22.50
LP 10^4	25.49	25.56	25.65	25.73
LP 10^3	29.04	29.09	28.97	29.04
LP 10^2	32.11	32.09	32.13	32.50
LP 10^1	36.38	36.06	36.16	36.20

X. Troubleshooting

- Amplification from the non-template control (NTC)

Cause	Solution
Formation of primer dimer	On the melting curve analysis, a peak at a temperature lower than that of the target peak suggests a primer dimer. The PCR cycle should be optimized. If the result is not improved, the following should be performed: change the primer sequence and/or change the purification grade of the primer (HPLC grade).
Contamination or carry-over of the PCR products	When the no-template control generates a peak at the same melting temperature as the target on the melting curve analysis, the amplification is caused by a carry-over or contamination. Use fresh reagents.

- Low fluorescence intensity

Cause	Solution
Incorrect handling	dsDNA-binding fluorescent dye is light sensitivity; avoid exposure to light and repeated freeze-thaw cycles. Always thaw and mix solutions thoroughly before use.
Incorrect concentration of ROX reference dye	If an incorrect concentration of ROX reference dye is added to the master mix, the normalized signal may be lower than expected (if too much ROX has been added), or higher than expected (if too little ROX has been added). If using ABI instrumentation, analysis of the raw signal can always be performed with the ROX filter switched off.

XI. Ordering Information

Cat. No.	Product Description
K-6251	*AccuPower*® 2X *GreenStar*™ qPCR MasterMix with 80X ROX Dye / 100Rxn, 50 µL reaction
K-6252	*AccuPower*® 2X *GreenStar*™ qPCR MasterMix with 80X ROX Dye / 200Rxn, 50 µL reaction
K-6253	*AccuPower*® 2X *GreenStar*™ qPCR MasterMix / 100Rxn, 50 µL reaction
K-6254	*AccuPower*® 2X *GreenStar*™ qPCR MasterMix / 200Rxn, 50 µL reaction

- **Related products**

Cat. No.	Product Description
K-2101	*AccuPower*® *RocketScript*™ RT Premix
K-3032	*AccuPower*® Genomic DNA extraction kit

XII. Notice

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