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2x SYBR Green qPCR Master Mix

Description

Bimake™ 2x SYBR Green qPCR master mix utilizes a special performance-enhanced Taq DNA polymerase protected via a hot-start activation technique, and optimized qPCR buffer system to perform SYBR Green I based quantitative PCR (qPCR). The mix, prepared at 2x reaction concentration, can be directly used for robust and low-template quantitative PCR with high sensitivity, specificity and reliability.

Components

Component	Cat #: B21202	Cat #: B21203
Bimake™ 2x SYBR Green qPCR Master Mix[a]	5 ml (for 200 reactions with 50 µl/rxn)	25 ml (for 1000 reactions with 50 µl/rxn)
50× ROX Reference Dye 1[b]	200 µl	1000 µl
50× ROX Reference Dye 2[b]	200 µl	1000 µl

a. Contain hot-start DNA Polymerase, dNTPs, Mg^{2+}, and SYBR Green I dye.

b. Used to rectify the error of fluorescence signals between different wells.

Storage

• All reagents should be stored at -20 °C for 2 years.

Notice

Please select appropriate ROX according to the Real-time PCR instrument used:

DO NOT USE ROX Reference Dye	Bio-Rad CFX96™, CFX384™, iCycleriQ™, iQ™5, MyiQ™,Opticon®, Opticon 2, Chromo4™; MiniOpticon™, Cepheid SmartCycler®; Eppendorf Mastercycler® eprealplex, realplex 2s; Illumina Eco qPCR; Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000; Roche Applied Science LightCycler™ 480; Thermo Scientific PikoReal Cycler.
USE ROX Reference **Dye 1 (high Conc.)**	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOne Plus™.
USE ROX Reference **Dye 2 (low Conc.)**	Applied Biosystems 7500, 7500 Fast, ViiA™7; Stratagene MX4000™, MX3005P™, MX3000P™.

Protocol

1. Guidance for qPCR Amplification

1). The suggested template amount is 10 to100 ng for genomic DNA or 1 to 10 ng for cDNA template. The following loading table is designed for reaction volume of both 50 µL and 20 µL.

Component	Amount per reaction (µl)	Amount per reaction (µl)	Final concentration
2x Bimake™ SYBR Green Master Mix	25	10	1x
Template	Variable	Variable	1 to 100 ng
Forward Primer (5 µM)	0.5 - 9	0.2 - 3.6	50 to 900 nM
Reverse Primer (5 µM)	0.5 - 9	0.2 - 3.6	50 to 900 nM
ROX Reference Dye	1	0.4	1x
Distilled Water (dH_2O)	Add to 50	Add to 20	-
Total Reaction Volume	50	20	-

2). Standard 2-step amplification program:

Step	1	2		3		
	Hot-Start DNA Polymerase Activation	PCR		Melt Curve		
	HOLD	CYCLE (40 cycles)		CYCLE (1 cycle)		
		Denatur	Anneal / Extend			
Temp.	95.0 °C	95.0 °C	60.0 °C	95.0 °C	60.0 °C	95.0 °C
Time	5 - 10 mins	15 secs	30 - 60 secs	15 secs	60 secs	15 secs
Volume	50 µL			50 µL		

3). Fast 2-step amplification program:

Step	1	2		3		
	Hot-Start DNA Polymerase Activation	PCR		Melt Curve		
	HOLD	CYCLE (40 cycles)		CYCLE (1 cycle)		
		Denatur	Anneal / Extend			
Temp.	95.0 °C	95.0 °C	60.0 °C	95.0 °C	60.0 °C	95.0 °C
Time	5 - 10 mins	3 secs	15 - 30 secs	15 secs	60 secs	15 secs
Volume	50 µL			50 µL		

IMPORTANT: The 5 -10 mins, 95 °C step is required to activate the Hot-Start DNA Polymerase.

Order & Inquiry
Tel: (713)732-2181
Fax: +1-866-747-4781
E-mail: order@bimake.com

Order & Inquiry
Tel: +49-89-46148500
Fax: +49-89-461485022
E-mail: eu.order@bimake.com

Data Analysis

Relative quantitation: Target gene expression is measured against an internal standard.

Gene expression can be measured by the quantitation of cDNA converted from a messenger RNA corresponding to this gene relative to a calibrator sample serving as a physiological reference. In a typical experiment, gene expression levels are studied as a function of either a treatment of cells in culture, of patients, or of tissue type.

The calibrator sample in each case is the cDNA from either the untreated cells or patients, or a specific tissue type. All quantitations are also normalized to an endogenous control such as 18S rRNA to account for variability in the initial concentration and quality of the total RNA and in the conversion efficiency of the reverse transcription reaction.

Set Ct^{A1} as the Ct value of the target gene of sample 1, and Ct^{B1} as the Ct value of the internal control gene of sample 1; Set Ct^{A2} as the Ct value of the target gene of sample 2, and Ct^{B2} as the Ct value of the internal control gene of sample 2. The expression difference (in fold) of the target gene in sample 1 and in sample 2 can be calculated this way ($2^{-\Delta\Delta Ct}$ approach):

$$\Delta\Delta Ct = (Ct^{A2} - Ct^{B2}) - (Ct^{A1} - Ct^{B1})$$

The expression level of the target gene in sample 2 is $2^{-\Delta\Delta Ct}$ times that of sample 1.

2) **Absolute quantitation:** Compares the Ct of an unknown sample against a standard curve with known copy numbers.

   Absolute quantitation is applicable only if isolation procedure and sample contents do not effect PCR amplification. The quantitation of genomic DNA may lend itself for absolute quantitation against a standard curve.

3) **Passive Reference ROX:** is a dye molecule included in the SYBR Green PCR Master Mix that does not participate in the PCR amplification. On Applied Biosystems real-time PCR systems, the passive reference provides an internal reference to which the SYBR Green / dsDNA complex signal can be normalized during data analysis. Normalization is necessary to correct for well-to-well fluorescent fluctuation.

4) **Determination of baseline and Threshold:** Please refer to the real-time PCR system software used to calculate baseline and threshold values for a detector or manually set it up according to amplification curves (Fig 1).

Trouble Shooting

Problem	Potential Cause(s)	Suggestion(s)
Apparent amplification can be observed in negative control	The reagents or water used is contaminated	Change new reagents or water and retry. The reaction should be set up in a super clean bench to minimize contamination from the air.
	Appearance of primer dimer	It is normal that amplification occurs in negative control after 35 cycles. Analysis can be performed combining with the melt curve.
Ct value appears too late(high)	Low amplification efficiency	Optimize the reaction. Try three-step program or re-design the primers.
	The concentration of templates is too low	Scale up administration dose in the case of exclusion of animal lethality.
	Degradation of templates	Prepare new templates and retry.
	The amplicon is too long	The length of the amplicon is recommended to be within 100 bp-200 bp.
	There are PCR inhibitors in the reaction	Inhibitors are usually brought in when adding templates. Increase the dilution folds or prepare new templates and retry.
Abnormal shape of amplification plot	Rough amplification plot	It is caused by system rectification due to weak signal. Elevate the template concentration and repeat the reaction.
Abnormal shape of amplification plot	Broken or downward amplification plot	The concentration of templates is too high. The end value of the baseline is bigger than Ct value. Decrease the end of the baseline (Ct value - 4), and re-analyze the data.
	Amplification plot goes downward suddenly	There are bubbles left in the reaction tube, which break up when the temperature rises, thus the instrument detects the sudden decrease of the fluorescence value. Spin briefly and check closely if there are bubbles left before reaction.
No amplification plot	Cycling number is insufficient	Generally the cycling number is set to be 40. But notice that too many cycles will result in excessive background, thereby reducing the reliability of the data.
	Check if there is signal collection procedure during cycling	In two-step program, signal collection is usually positioned at annealing and extension stage; for three-step program, signal collection should be positioned at 72 °C extension stage.
	Check if the primers are degraded	Test the integrity of primers after long-term storage through PAGE electrophoresis to confirm if the primers are degraded already.
	The concentration of templates is too low	Reduce the dilution fold and retry. For target gene with unknown expression level, begin without template dilution.
	Degradation of templates	Prepare new templates and retry.

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