2x SYBR Green qPCR Master Mix (Low ROX)

The 95 °C step for 5 minutes is required to activate the Hot-Start DNA Polymerase.

1. General Information

Bimake[TM] 2x SYBR Green qPCR master mix (Low ROX) 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.

2. Package Information

Component	Cat #: B21702	Cat #: B21703
Bimake™ 2x SYBR Green qPCR Master Mix (Low ROX)[a]	5 ml (for 200 reactions with 50 µl/rxn)[b]	25 ml (for 1000 reactions with 50 µl/rxn)

[a] Contain hot-start DNA Polymerase, dNTPs, Mg^{2+}, SYBR Green I dye and low concentration of ROX.

[b] 10 µl and 20 µl of reaction volumes are also applicable using this product.

This product is specifically designed for the following instruments:

Use Low Conc. of ROX	Applied Biosystems 7500, 7500 Fast, ViiA™7; Stratagene MX4000™, MX3005P™, MX3000P™.

When the following instruments are used, please choose Bimake SYBR Green Master Mix with Cat # : B21202, B21203.

DO NOT USE ROX	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.

3. Storage

Stored at -20 °C for 2 years.

4. 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[TM] SYBR Green Master Mix (Low ROX)	25	10	1x
Template	Variable	Variable	1 to 100 ng
Forward Primer (5µM)	0.5 - 9	0.2 - 3.6	50 to 900 nM
Reverse Primer (5µM)	0.5 - 9	0.2 - 3.6	50 to 900 nM
Distilled Water (dH_2O)	Add to 50	Add to 20	-
Total Reaction Volume[a]	50	20	-

[a] 10 µl of reaction volume can be set up proportionally using this product.

2) Standard two-step amplification program:

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

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

5. Data Analysis

Two types of quantification methods are commonly applied to quantify target gene expression when using this master mix.

A typical amplification curve, as shown below , has a:

- Plateau phase (a)
- Linear phase (b)
- Exponential (geometric phase) (c)
- Background (d)
- Baseline (e)

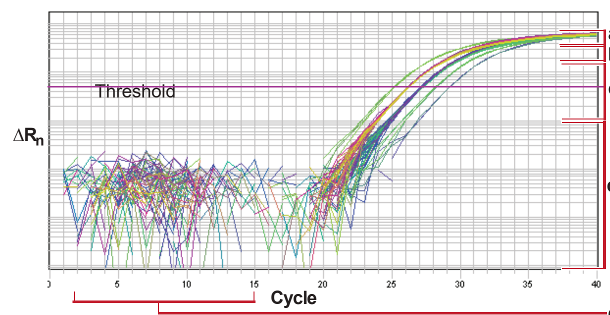

Figure1: Amplification curve

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Note:
Quantitation is most accurate when Ct value is located between 20 and 28. If the Ct value is too small ($<$15), please dilute the template and repeat the quantitation; if the Ct value is higher than 35, the amplification is invalid; if the Ct value is between 32 and 35, 3 replicates are required to validate the results.

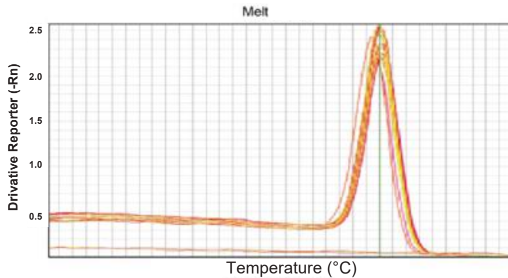

Figure2: Unimodal melt curve

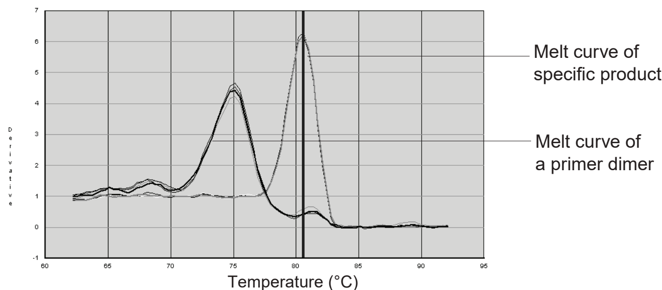

Figure3. Multimodal melt curve

Note:
A typical valid dissociation curve should be unimodal due to highly specific amplification and no primer dimer formation (Fig 2). If the dissociation curve is apparently multimodal (Fig 3), it often suggests primer dimmer formation, non-specific amplification, or other contamination.

Alternatively, confirmation of non-specific amplification can be analyzed by agarose gel electrophoresis.

1) 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).

6. Troubleshooting

1) Abnormal shape of amplification plot

a) Rough amplification plot: It is caused by system rectification due to weak signal. Elevate the template concentration and repeat the reaction.
b) Broken or downward amplification plot: The concentration of templates is too high. The end value of the baseline is bigger than Ct value.
c) 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.

2) No amplification plot

a) 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.
b) 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.
c) Check if the primers are degraded: Test the integrity of primers after long-term storage through PAGE electrophoresis to confirm the presence of the primers in solution.
d) The concentration of templates is too low: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without template dilution.
e) Degradation of templates: Prepare new templates and retry.

3) Ct value appears too late (high)

a) Low amplification efficiency: Optimize the reaction. Try three-step program or re-design primers.
b) The concentration of templates is too low: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without template dilution.
c) Degradation of templates: Prepare new templates and retry.
d) The amplicon is too long: The length of the amplicon is recommended to be within 100 bp - 200 bp.
e) There are PCR inhibitors in the reaction: They are usually brought in when adding templates. Increase the dilution folds or prepare new templates and retry.

4) Apparent amplification can be observed in negative control

a) The reagents or water used is contaminated: Change to new reagents or water and retry. The reaction should be set up in a clean bench to minimize contamination from the air.
b) 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.

5) Pre-denaturation / enzyme activation time

This mix is based on a hot-start DNA polymerase, the pre-denaturation / enzyme activation condition should be set to 95 °C for 5 minutes to thoroughly activate the enzyme. If the template is GC-rich, the pre-denaturation / enzyme activation time should be prolonged to 10 minutes.

7. MSDS

The MSDS information of this product can be downloaded from Bimake.com.

Note: The full version of the user manual can be viewed and downloaded on www.bimake.com

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