2x SYBR Green qPCR Master Mix

Components

Component	Cat #: B21202	Cat #: B21203
2x SYBR Green qPCR Master Mix[a]	5 mL (200 Rxns)	25 mL (1000 Rxns)[c]
50× ROX ReferenceDye 1[b] (High Conc)	200 µL	1000 µL
50× ROX Reference Dye 2[b] (Low Conc)	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. It needs to be added according to different instrument models.

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	**Bio-Rad** CFX96™, CFX384™, iCycler iQ™, 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:** LightCycler® 480, 96, Nano, 1.5/2.0**; **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, QuantStudio 6 and 7 Flex System, QuantStudio 3 and 5; **Agilent Stratagene:** MX4000™, MX3005P™, MX3000P™.

Protocol

1. qPCR reaction system (Take 20 µL and 50 µL for examples):

Component	Amount per reaction (µL)	Amount per reaction (µL)	Final concentration
2x Selleck SYBR Green Master Mix	25	10	1x
Template[c]	Variable	Variable	1-100 ng
Forward Primer[d] (5 µM)	2.5	1	0.2-1 µM
Reverse Primer[d] (5 µM)	2.5	1	0.2-1 µM
ROX Reference Dye	1	0.4	1x
Distilled Water (dH_2O)	Add to 50	Add to 20	-
Total Reaction Volume[c]	50	20	-

c. The amount of template adding into the system varies with the number of copies of target genes. Gradient dilution is preferred to determine the optimum template addition, which generally does not exceed 10% of the total system. The reverse transcription product of 1 µg RNA (10 µL reverse system) is recommended to be diluted 10 times as the template. Usually, 1 µL is added to 20 µL qPCR system. For low abundance genes, the template quantity in qPCR system should be increased properly.

d. Generally, keep the final concentration of upstream and downstream primers at 0.5 µM to guarantee the best results. But when the reaction performance is poor, try to find the optimal primer concentration between 0.2 and 1 µM. If the amplification efficiency is too low, increase the primer concentration. If the reaction specificity is poor, reduce the primer concentration.

e. This product can also be used in 10 µL reaction system. For low abundance genes, it is recommended to use more than 20 µL reaction system.

2. qPCR program settings

This kit can be applied to two-step and three-step procedures, while the three-step method is better. When the reaction performance is poor, the following adjustments can be made. If the amplification efficiency is poor, the thermal start time can be prolonged, the annealing temperature can be reduced or the extension time can be increased. If the amplification specificity is poor, the annealing temperature can be increased appropriately.

	1	2		3		
Two step	Hot-Start DNA Polymer	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	30 sec -10 min[f]	15 sec	30-60 sec	15 sec	60 sec	15 sec
Volume	50 µL			50 µL		

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Three 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	50.0-60.0 °C	72.0 °C	95.0 °C	60.0 °C	95.0 °C
Time	30 sec-10 min[f]	15 sec	30 sec	30 sec	15 sec	60 sec	15 sec
Volume	50 μL				50 μL		

f. Heat for 30 sec-10 min at 95°C to activate the heat-activated DNA polymerase. For the amplified sequence which is rich in GC, the time needs increase to 10 min.

3. Result analysis

Amplification curve: Generally, the ranges of CT values are 15 to 35, while 20-28 are the best. If the CT value is too low, increase the dilution ratio of template. If the CT value is too high, raise the concentration of templates or primers, even adjust the qPCR program.

Melting curve: Usually only when the melting curve is single peak, the quantitative result can be qualified. If there are multiple peaks in the dissolution curve, it is necessary to optimize the conditions, such as redesign primers.

Trouble Shooting

Problem	Potential Cause(s)	Suggestion(s)
Amplification worked in the negative control	The reagent or de-ionized water used is contaminated	Use new reagents and de-ionized water when operating in a clean laboratory bench
	Primer dimers	It is normal to produce amplification curves in the negative control after 35 cycles. Please analysis the results according to their melting curves.
Ct is higher or lower than the normal values	Low amplification efficiency	Optimize the reaction system, try three-step method or redesign primers
	Low template concentration	Increase the concentration of template
	Template degradation	Prepare fresh template
	Too long amplification fragments	The length of amplification fragments should be 100-200 bp
	PCR inhibitors exist in reaction system	Try to dilute or re-prepare the template, because inhibitors were usually added with the template.
Abnormal amplification curves	Abnormal shape of the amplification curve	When the signal is weak, the system calibration may lead to this result, which can be corrected by increasing the template concentration.
	Fractured or descending shape of the amplification curve	When the template concentration is too high, the baseline endpoint value is higher than the CT value. Decrease the baseline endpoint value (Ct value minus 4) and re-analysis the data.
	Suddenly falling shape of the amplification curve	The bubbles in the reaction system will burst suddenly when the temperature rises. The equipment will detect a sudden drop in fluorescence value. Centrifuge and check whether there are bubbles to avoid this problem.
Without a amplification curve	Insufficient cycle number	The cycle number is usually set to be 40
	No 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
	The primer degradation	After long-term storage, the integrity of primers should be confirmed by PAGE gel
	The template concentration is too low	Decrease the dilution ratio (For target genes with unknown expression, their template was used without dilution for the first time)
	The template degradation	Prepare fresh template
Heterozygous peak of melting curve	Unreasonable primer design	The undesired peaks of primer dimers often occur at about 75°C. If the peak is significant, the primers need to be redesigned.
	The primer concentration is too high	Decrease primer concentration properly
	The template concentration is too low	Increase template concentration
	The contamination of genome DNA	Design primers by transcending introns
Poor stability of duplicated wells	Sampling error	Increase the reaction system; Increase the dilution ratio and sampling volume of templates
	The template concentration is too low	Increase the sample size
	Instrument problems	The temperature of each hole varies, so it is necessary to calibrate the instrument before use.