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FastReal qPCR PreMix (SYBR Green)

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Cat. no. 4995063/4995064/4995164

Kit Contents

Contents	4995063 20 µl × 125 rxn	4995064 20 µl × 500 rxn	4995164 20 µl × 5000 rxn
2 × FastReal qPCR PreMix (SYBR Green)	1.25 ml	4 × 1.25 ml	10 × 4 × 1.25 ml
50 × ROX Reference Dye	250 µl	1 ml	10 × 1 ml
RNase-Free ddH_2O	1 ml	5 × 1 ml	10 × 5 × 1 ml
Handbook	1	1	10 × 1

Storage Conditions:

FastReal qPCR PreMix (SYBR Green) can be stored at -30~-15°C for one year.

It should be stored immediately upon receipt at -30~-15°C. 2 × FastReal qPCR PreMix (SYBR Green I) and 50 × ROX Reference Dye should be thawed and then mixed upside down gently to be homogenous before using. If the reagents have been thawed but not used, it is important to thoroughly mix prior to re-freezing. (The layering of salts during the thawing process and subsequent crystallization during freezing will damage the enzyme and decrease product performance.) The reagents could be stored for up to 3 months at 2-8°C if frequently used. Please avoid refreezing and thawing repeatedly.

Introduction

FastReal qPCR PreMix (SYBR Green) is designed for SYBR Green I based quantitative PCR assays, enables highly sensitive, rapid and specific quantitative detection of target DNA. Optimized premix could reduce the running time and is suitable for regular and fast real-time PCR thermal cycler.

The hot-start Taq DNA polymerase used in the FastReal qPCR PreMix is modified with a novel antibody. Combined with the optimized Fast PCR buffer, it could ensure a sensitive PCR detection on any Real-Time PCR thermal cycler. It features fast reaction, high amplification efficiency, high amplification specificity, high sensitivity, early peak amplification curve and high fluorescence value, allowing you to obtain results faster and save research time and energy without compromising PCR results.

Reagent kit features

1. **Fast reaction.** The hot-start Taq DNA polymerase in the FastReal qPCR PreMix has short hot-start time, high enzyme activity and fast reaction; the components in the reaction buffer, after targeted modification, can greatly shorten the denaturation, annealing and extension time, which can save up to 50% of the reaction time and obtain the experimental results quickly.
2. **High amplification capacity.** The new hot-start Taq DNA polymerase, combined with the optimized Fast PCR buffer, ensures high amplification efficiency and product specificity of the reagent, and features early peak onset and high fluorescence value of the amplification curve.
3. **Transparent tube, reagent residual is clearly visible.** The 2 × FastReal qPCR PreMix is packaged in colorless transparent tubes with good light stability, and the transparent tubes are more convenient for customers to access.
4. **The instrument is widely applicable.** It is not only suitable for fast quantification instruments, but also for common quantification instruments.

Important Notes

1. When preparing PCR PreMix, attention should be paid to mixing well. If the reagents are not mixed well, it will lead to high local concentration of PCR components and make the reaction performance degraded.
2. The purity of primers is important for the specificity of PCR. Primers purified by PAGE or more superior methods are recommended.

3. Typically, best amplification results can be obtained using a primer concentration of 0.3 μM. However, for individual determination of optimal primer concentration, a primer titer from 0.2 μM to 0.5 μM can be performed.
4. In a 20 μl reaction volume, the amount of cDNA template is usually less than 100 ng, and genomic DNA is less than 50 ng. The reverse transcription product, if used as template, should not comprise more than 20% of the total PCR reaction volume.

Protocol

<1> Set up the Real-Time reaction

Note: 50 × ROX Reference Dye should be stored protected from light.

1. Thaw 2 × FastReal qPCR PreMix (if stored at -30~-15°C), ROX Reference Dye, template, primers, and RNase-Free ddH_2O. Completely mix and equilibrate all the reagents to room temperature before use.
2. Prepare reaction solution according to the following table. All the steps should be operated on ice.

Reaction system:

Component	50 μl volume	25 μl volume	20 μl volume	Final concentration
2 × FastReal qPCR PreMix (SYBR Green)	25 μl	12.5 μl	10 μl	1 ×
Forward primer (10 μM)	1.5 μl	0.75 μl	0.6 μl	300 nM*
Reverse primer (10 μM)	1.5 μl	0.75 μl	0.6 μl	300 nM*
cDNA template	—	—	—	-ng-pg
50 × ROX Reference Dye △	—	—	—	—
RNase-Free ddH_2O	Up to 50 μl	Up to 25 μl	Up to 20 μl	—

* A final primer concentration of 0.3 μM is optimal for most applications. Higher concentration can be used when the amplification efficiency is not favorable. If non-specific amplification is observed, the primer concentration should be decreased. For further optimization, a primer titration from 0.2 μM to 0.5 μM can be performed.

△ The optimal concentration of ROX Reference Dye for commonly used Real-Time PCR thermal cycler:

Instrument	Final concentration
ABI 7000/7300/7700/7900/7900HT/7900HT Fast, StepOne™/StepOne Plus™	5 × (e.g., 5 μl ROX/ 50 μl volume)
ABI 7500/7500 Fast, ViiA 7, QuantStudio™ 3 /5/6 Flex/7 Flex/12K Flex; Agilent Stratagene Mx3000P/Mx3005P/Mx4000	1 × (e.g., 1 μl ROX/ 50 μl volume)
Instruments of Roche, Bio-Rad and Eppendorf etc.	No need

<2> Real-Time PCR

Typically, optimal results are obtained using a two-step PCR. Three-step PCR could be considered if low amplification efficiency due to low copies of template is observed.

Two-step PCR procedure

Stage	Cycle	Temperature	Time	Step	Signal Collection
Initial denaturation	1 ×	95°C	2 min	Initial denaturation	N
PCR	40 ×	95°C	5 sec	Denaturation	N
		60°C △1	15 sec △2	Annealing/ extension	Y
Melting/Dissociation Curve Stage					

Three-step PCR procedure

Stage	Cycle	Temperature	Time	Step	Signal Collection
Initial denaturation	1 ×	95°C	2 min	Initial denaturation	N
PCR	40 ×	95°C	5 sec	Denaturation	N
		50-60°C △3	10 sec	Annealing	N
		72°C	15 sec △2	Extension	Y
Melting/Dissociation Curve Stage					

△1 60°C for 15 sec is optimal for most application. For further optimization, please try 56-66°C.

△2 Set time according to different instruments requirement. The optimal annealing and extension time for commonly used Real-Time PCR instruments is as below:

ABI 7700/7900HT/7500 Fast, Roche, BioRad and Agilent etc: 15 sec.
ABI 7000/7300: 31 sec.
ABI 7500: 32 sec.

△3 Annealing temperature of primers is usually 5°C lower than its melting temperature (Tm). The annealing temperature could be increased properly if the base number is low, which could increase the specificity. On the contrary, the annealing temperature could be decreased if the base number is high.

3. Close the tubes and mix samples gently. Briefly centrifugation can be performed to collect residual liquid from the walls of the tubes.
4. Place the PCR tubes in the thermal cycler and then start the PCR cycle.