TIANGEN®

Talent qPCR PreMix (SYBR Green)

For fast, universal, quantitative, real-time PCR using SYBR Green

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Talent qPCR PreMix (SYBR Green)

Cat. no. GFP209

Kit Contents

Contents	GFP209-01 20 μl×125 rxn	GFP209-02 20 μl×500 rxn	GFP209-03 20 μl×5000 rxn
2× Talent 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

The Talent qPCR PreMix (SYBR Green) Kit can be stored at -30~-15°C for two years. It should be stored at -30~-15°C immediately after receipt, protected from light. Thaw the 2×Talent qPCR PreMix (SYBR Green) and 50×ROX Reference Dye and mix thoroughly before use. If the 2×Talent qPCR PreMix (SYBR Green) and 50×ROX Reference Dye are thawed but not used, it is important to thoroughly mix them prior to refreezing. The delamination of salts during the thawing process and subsequent crystallization during freezing will damage the enzyme and decrease product performance. For frequent use, 2×Talent qPCR PreMix (SYBR Green) can be stored at 2-8°C for 3 months. Repeated freeze-thaw cycles should be avoided.

Introduction

The Talent qPCR PreMix (SYBR Green) Kit is designed for SYBR Green I based quantitative PCR assays, which enables fast and specific quantitative results. Optimized premix could reduce the running time and is suitable for regular and fast real-time PCR thermal cycler.

2×Talent qPCR PreMix (SYBR Green) adopts antibody modified Anti *Taq* DNA polymerase. Combined with the unique PCR buffer, it could ensure a sensitive PCR detection on any Real-Time PCR thermal cycler. Total running time could be reduced by 50% compared to regular real-time PCR program. In addition, the H-competitor factor and EP components added in the PreMix make the product have good sample universality especially for templates with complex secondary structures, with long PCR products and with more PCR inhibitors. Meantime accurate quantification, high amplification efficiency, high specificity and wide credibility range could be achieved.

Important Notes

1. The 2×Talent qPCR PreMix adopts anti-*Taq* DNA polymerase modified by antibodies, which is combined with special fast qPCR Buffer system that can greatly reduce denaturation, annealing and extension time, and save up to 50% reaction time.
2. The H-Competitor factor is specially added in the PreMix, which could compete the hydrogen bond with DNA, and could open the DNA double strand easily, making the products have good sample universality, especially for templates with complex secondary structures, with long PCR products and with more PCR inhibitors.
3. The special fast PCR Buffer system of this product contains the unique PCR stabilizer, EP, which can effectively protect the enzyme's activity, resist the interference of various PCR inhibitors, and ensure the high amplification efficiency, high amplification sensitivity and wide range of reliability of 2×Talent qPCR PreMix.
4. 2×Talent qPCR PreMix is premixed with SYBR Green I. When the PCR reaction solution is prepared, the rapid real-time PCR reaction can be carried out simply by adding template, primer and sterilized distilled water. The operation is simple and convenient.
5. This product comes with ROX Reference Dye, which can eliminate signal background and calibrate the fluorescent signal error between different wells. It is convenient for the customer to choose the corresponding concentration depend on different real-time PCR systems.

6. 2×Talent qPCR PreMix is packaged with colorless transparent tubes. After detection, lighting will not affect the quantitative results of the system.

Principle of Operation

This product uses antibody-modified hotstart DNA polymerase for rapid PCR amplification, and the amplified PCR products are quantified by detecting the fluorescence intensity of SYBR Green I in the reaction process. It is suitable for regular and fast real-time PCR thermal cycler.

1. Antibody modified hotstart *Taq* has a short activation time, the denaturation, annealing and elongation time can be greatly reduced and meanwhile avoiding the amplification of nonspecific products. This product can save up to 50% reaction time, and get experimental results more quickly.
2. The H-competitor factor and optimized PreMix make the products have good sample universality especially for templates with complex secondary structures, with long PCR products and with more PCR inhibitors.
3. In view of the differences in the structure of cDNA template and gDNA template, this product is specially optimized for system reaction steps of PCR, so that gDNA template which is difficult to be amplified can also obtain good PCR results.

Important Notes

1. Gently mix the reagents by inverting the tubes and centrifuge briefly prior to use. Do NOT vortex and avoid producing bubble.
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. If reverse transcribed products are used as template, the template input should be no more than 20% of the total PCR reaction volume.

Protocol

<1> Set up the Real-Time PCR reaction system

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

1. Fully thaw RNA template, primers, 2×Talent qPCR PreMix (SYBR Green), 50×ROX Reference Dye and RNase-Free ddH_2O. Centrifuge transiently and put all of them on ice.
2. Prepare a reaction solution accord to the following table (All the steps should be operated on ice).

Component	50 µl	25 µl	20 µl	Final concentration
2×Talent 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	0.3 µM*
Reverse Primer (10 µM)	1.5 µl	0.75 µl	0.6 µl	0.3 µM*
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. However, for individual determination of optimal primer concentration, a primer titration from 0.2-0.5 µM can be performed. Higher primer concentration will increase the amplification efficiency, but if non-specific amplification is observed, the primer concentration should be reduced.

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

Instrument	Final Concentration
ABI PRISM 7000/7300/7700/7900HT/ StepOne etc.	5× (e.g. 5 µl ROX/ 50 µl volume)
ABI 7500 Fast, 7500 Fast, ViiA7, Stratagene Mx3000P, Mx3005P, Mx4000 etc.	1× (e.g. 1 µl ROX/ 50 µl volume)
Instruments of TGreat Real 96, Roche, Bio-Rad and Eppendorf, etc.	No need

<2> Real-Time PCR

Typically, two-step PCR is recommended. However, if two-step PCR does not yield favorable results (e.g. non-specific amplification caused by low template concentration or reduced amplification efficiency induced by low Tm value) three-step PCR is recommended.

Two-step PCR program:

Stage	Cycle	Temperature	Time	Step	Signal Collection
Initial denaturation	1×	95°C	3 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 program:

Stage	Cycle	Temperature	Time	Step	Signal Collection
Initial denaturation	1×	95°C	3min	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 An Annealing/Extension temperature of 60°C for 15 sec is optimal for most applications. However, if further optimization on reaction specificity is required, temperature from 56°C to 66°C can be performed.

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

TGreat Real 96 /ABI 7700/7900HT/7500 Fast, ViiA 7, Roche, BioRad and Agilent etc: 15 sec
ABI 7000/7300: 31 sec
ABI7500: 32 sec

△[3] Annealing temperature of primers is usually 5°C lower than its melting temperature (Tm). The annealing temperature could be increased properly.

3. Close the tubes and mix samples gently. Brief 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.