TOOLS SuperFast SYBR qPCR Reagent

Cat. no.: FPT-BB07
Storage: Store at −20°C
Product Size: 125 rxn, 20 µL per rxn

Introduction

The TOOLS SuperFast SYBR qPCR Reagent is designed to yield fast and detailed quantitative results in SYBR Green I–based quantitative PCR assays. The optimized premix potentially reduces running time and is suitable for use in a regular and fast real-time PCR thermal cycler. The 2 × SuperFast SYBR qPCR Mix uses an antibody-modified anti-taq DNA polymerase. Combined with the unique PCR buffer, the Reagent ensures sensitive PCR detection for any real-time PCR thermal cycler. Total running time can be reduced by 60% relative to regular real-time PCR programs, and accurate quantification, high amplification efficiency, high specificity, and a wide credibility range can be achieved.

Important Notes

1. The TOOLS 2 × SuperFast SYBR qPCR Mix includes SYBR Green I. Store the reagent in the dark and avoid direct exposure to strong light during the preparation of PCR reaction mixtures.
2. Gently mix the reagents by inverting the tubes and centrifuge briefly prior to use. Do *not* vortex to prevent bubbles from forming.
3. The purity of primers is important for the specificity of PCR. Primers purified by PAGE (or superior methods) are recommended.
4. Typically, the best amplification results can be obtained using a primer of 0.3-µM concentration. However, for the individual determination of optimal primer concentration, a primer titration from 0.2 µM to 0.5 µM can be performed.
5. For a reaction volume of 20 µL, the amounts of genome DNA or cDNA template are usually less than 100 ng. The reverse transcription products, if used as template, should not comprise more than 20% of the total PCR reaction volume.

Contents	FPT-BB07 (125 rxn, 20 µL per rxn)
2X SuperFast SYBR qPCR Mix	1.25 mL
50×ROX Dye	250 µL
RNase free ddH_2O	2 x 1 mL

Storage

The TOOLS SuperFast SYBR qPCR Reagent should be stored immediately upon receipt at −20°C. The 2 × SuperFast SYBR qPCR Mix and 50 × ROX Dye should be thawed and then mixed upside down gently to ensure homogeneity before use. If the reagents have been thawed but not used, it is important to mix them thoroughly before refreezing; the layering of salts during the thawing process and subsequent crystallization during freezing will damage the enzyme and decrease product performance. The reagents can be stored for up to 3 months at 2°C–8°C if frequently used. Please avoid repeated thawing and refreezing.

Protocol

A. Setup of the real-time reaction system

Note: The 2 × SuperFast SYBR qPCR Mix and 50 × ROX Reference Dye should be stored in a place that is protected from light.

1. Thaw the 2 × SuperFast SYBR qPCR Mix (if stored at −20°C), 50 × ROX Reference Dye, template, primers, and RNase-free ddH_2O. Completely mix and equilibrate the reagents to room temperature before use.

2. Prepare a reaction solution according to the following table. All steps should be executed on ice.

Component	50 µL volume	25 µL volume	20 µL volume	Final concentration
2X SuperFast SYBR qPCR Mix	25 µL	12.5 µL	10 µL	1×
Forward Primer (10µM)	1.5 µL	0.75 µL	0.6 µL	0.3 µM[*1]
Reverse Primer (10µM)	1.5 µL	0.75 µL	0.6 µL	0.3 µM[*1]
cDNA template	-	-	-	-ng-pg
50×ROX Dye[*2]	-	-	-	-
RNase-free ddH_2O	Up to 50 µL	Up to 25 µL	Up to 20 µL	-

a. A final primer concentration of 0.3 µM is optimal for most applications. Higher concentrations

can be used when amplification efficiency is not favorable. If nonspecific amplification is observed, however, the primer concentration should be reduced. For further optimization, primer titration from 0.2 μM to 0.5 μM can be performed.

b. Optimal concentration of ROX Dye for commonly used real-time PCR instruments:

Instrument	Final concentration
ABI PRISM 7000/7300/7700/7900HT/Step one	5× (e.g. 5 μL ROX/50 μL volume)
ABI 7500, 7500 Fast; Stratagene Mx3000P, Mx3005P and Mx4000	1× (e.g. 1 μL ROX/50 μL volume)
Instruments of Roche, Bio-Rad and Eppendorf	No need to add

B. Real-time amplification

Typically, two-step PCR yields the best results. However, if two-step PCR does not yield favorable results (due to, for example, nonspecific amplification from low template concentration or reduced amplification efficiency from a low Tm value), three-step PCR is recommended.

Two-step PCR

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

Three-step PCR

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

a. 60°C for 15 s is optimal for most applications. For further optimization, please try a temperature of 56°C–66°C.

b. Set the running time according to the requirements of the instrument. The optimal annealing and extension times for commonly used real-time PCR instruments are as follows

ABI7700/7900HT/7500 Fast, Roche, BioRad and Agilent etc	15s
ABI 7000/7300	31s
ABI7500	32s

c. The annealing temperature of primers is usually 5°C lower than its melting temperature (Tm). The annealing temperature can be appropriately increased.

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

Troubleshooting

No signals are detected, signals are detected late in PCR, or only primer–dimers are detected

Comments	Suggestions
Inhibitors in template	Reduce the template amount. If necessary, perform the purification procedure again.
Mg^{2+} concentration not optimal	The Mg^{2+} concentration in the provided 2 × SuperFast SYBR qPCR Mix is 2 mM. If the targets are few, an increase of <5 mM of Mg^{2+} may be helpful. Perform the titration in 0.5-mM steps.
Pipetting error or missing reagent	Check the concentrations and storage conditions of the reagents, including primers and template nucleic acids. Repeat the PCR.
HotStarTaq DNA Polymerase not activated	Ensure that the cycling program includes the initial denaturation step (15 min at 95°C) to activate the hot-start enzymes.
PCR programs or primer concentration not optimal	Use optimal primer concentrations and check for possible primer degradation. Modify the PCR thermal cycling according to the information provided in this handbook. If necessary, redesign the primers.
Problems with the starting template	Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acids from the stock solutions. Repeat the PCR using the new dilutions.

High fluorescence in the “No Template” control

Comments	Suggestions
Contamination of reagents	Discard reaction components and repeat the PCR with new reagents.
Contamination during reaction setup	Take appropriate safety precautions (e.g., use filter tips).
Primer degradation	Check for possible degradation of primers on a denaturing polyacrylamide gel.

Primer–dimers and/or nonspecific PCR products

Comments	Suggestions
Mg^{2+} concentration not optimal	The Mg^{2+} concentration in the provided TOOLS 2 × SYBR qPCR Mix is 2 mM. If the targets are few, an increase of <5 mM of Mg^{2+} may be helpful. Perform the titration in 0.5-mM steps.
Annealing temperature too low	Increase the annealing temperature in 2°C increments.
Primer design not optimal	Review the primer design.
PCR product too long	For optimal results, PCR products should be between 100 and 150 bp. PCR products should not exceed 500 bp.
Primers degraded	Check for possible degradation of primers on a denaturing polyacrylamide gel.
Metering inaccuracies	An overly small reaction volume may reduce detection accuracy. Use the manual-recommended volume and repeat the PCR.

No linearity in ratio of the CT value, or crossing point, to the logarithm of the template amount

Comments	Suggestions
Instrument malfunction	Operate the real-time PCR instrument according to the manual's instructions.
Contamination of templates	Contamination of templates may lead to poor linearity.
Long stored dilutions of template	Make new serial dilutions of template nucleic acids from the stock solutions. Repeat the PCR using the new dilutions.
PCR programs or primer concentration not optimal	Use optimal primer concentrations and check for possible primers degradation. Modify the PCR thermal cycling according to the information provided in this handbook. If necessary, redesign the primers.
Metering inaccuracies	An overly small reaction volume can reduce detection accuracy. Use the manual-recommended volume and repeat the PCR.