TOOLSpeed Quant SYBR Green Master mix

Cat. no.: ST-QS01

Storage:

Please store the reagent at -20 ℃ for long term storage and 4℃ for a short period (~2 weeks). Store in the dark and avoid direct exposure to strong light. e avoided.

Product Size:

Contents	ST-QS01 (100 rxn/20 μL)
TOOLSpeed Quant SYBR Green master mix	1 mL

Introduction

TOOLSpeed Quant SYBR Green master mix is an optimized 2X SYBR Green qPCR reagent that premixed low ROX reference dye. This 2X SYBR Green master mix is a high fluorescent and powerful reagent for real-time PCR in detecting nucleic acid samples by fluorescence signal accumulation. All the required components of qPCR were contained except specific primers and template. TOOLSpeed Quant SYBR Green master mix suited for qPCR instruments that required Low ROX mode, including ABI QuantaStudio series and the earlier version of ABI qPCR instruments.

Important Notes

1. TOOLSpeed Quant SYBR Green master mix includes SYBR Green I. Please 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 spin down prior to use. Do not vortex to prevent bubbles from forming. After usage, place the reagent back to -20 ℃ for long time storage or 4 ℃ for short period usage.
3. TOOLSpeed Quant SYBR Green master mix premixed with low ROX dye, and it suits for qPCR instruments that required Low ROX mode.
4. Typically, the best amplification results can be obtained using a primer of 0.2 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 suggested amounts of genome DNA or cDNA template between 10 pg to 50 ng. And a no template control group is suggested.
6. To ensure the confidence of experiment, at least 2 repeats of each sample is suggested.

Protocol

A. Set up the real-time reaction system

Note: The 2× SYBR PreMix and 50×ROX Dye should be stored and protected from light.

1. Thaw the TOOLSpeed Quant SYBR Green master mix (if stored at −20°C), primers, gDNA/cDNA template, and RNase-free ddH2O. 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	20 µL volume	10 µL volume*	Final concentration
TOOLSpeed Quant SYBR Green master mix	10 µL	5 µL	1 X
Forward Primer (10 µM)	0.4 µL	0.2 µL	0.2 µM
Reverse Primer (10 µM)	0.4µL	0.2 µL	0.2µM
template	X µL	X µL	-
RNase-free ddH_2O	Up to 20µL	Up to 10µL	-

* Setup of 10 µL volume suited for 384 well plate

B. Real-time PCR amplification

Two program were provided as following table. Normally, quantitative real-time PCR with optimal program yields the best results. However, if PCR results that were amplified with QuantStudio instrument does not yield favorable results, QuantStudio default program is recommended.

Optimal Program

Optimal program			
Stage	**Temperature**	**Time**	
Denaturation	95 ˚C	3 min	
Cycles	95 ˚C	5 sec	40 cycles
	60 ˚C	30 sec	
Melting Curve	default		

QuantStudio default program

QuantStudio default program			
Stage	**Temperature**	**Time**	
UDG activation	50 ℃	2 min	
Denaturation	95 ℃	2 min	
Cycles	95 ℃	1 sec	40 cycles
	60 ℃	30 sec	
Melting Curve	default		

3. Close the tubes and gently mix the samples. Quick spin-down 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 Guide

No amplification curve and no signals after reaction	
Comments	**Suggestions**
Inhibitors in template	Reduce the template amount. If necessary, perform the purification procedure again.
Pipetting error or missing reagent	Check the concentrations and storage conditions of the reagents, including primers and template nucleic acids. Repeat the PCR.
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**
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 70 and 300 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.