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2×SYBR Green qPCR Master Mix

Catalog Number: D026-1, D026-2

Table 1. Kit Components and Storage

Kit Component	D026-1	D026-2	Storage	Stability
2×SYBR Green qPCR Master Mix	5×1 mL	10×1 mL	-20 °C, avoid repeated freeze-thaw	The product is stable for one year when stored as directed.
ROX Reference Dye	100 µL	200 µL		

Product Description

ABP 2×SYBR Green qPCR Master Mix provides a convenient, reliable and robust setup for performing quantitative real-time analysis of DNA samples. This ready-to-use qPCR Master Mix contains HotStart *Taq* DNA Polymerase, dNTP, Mg^{2+}, stabilizer, enhancer and an optimized buffer system, which provides rapid extension and robust performance. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. It can be used for the quantitative detection of target sequences in genomic DNA or cDNA target after RNA reverse transcription. The SYBR Green I in the Master Mix will bind to double-stranded DNA, and can dissociate from DNA during the denature step. Based on this principle, the specificity of the amplification product can be determined using a melting curve.

HotStart *Taq* DNA Polymerase has 5'→3' polymerase activity and 5'→3' exonuclease activity, lacks 3'→5' exonuclease activity, and produces 3'-dA-tailed amplicons. qPCR products made with HotStart *Taq* DNA Polymerase can be used with TA cloning vectors. The SYBR Green qPCR Master Mix provides reproducible, sensitive and specific quantification of genomic, plasmid, viral, and cDNA templates. The SYBR Green qPCR Master Mix is compatible with most real-time thermal cyclers.

Special Features

- Specificity: HotStart *Taq* DNA polymerase and the optimized buffer eliminate non-specific amplification and formation of primer dimers.
- Sensitivity: Detects low copy number targets.
- Wide linear range: Accurate quantification across 9 orders of magnitude.
- Reproducibility and convenience: Ready-to-use 2× master mix.

Applications

- Gene-expression analysis.
- siRNA validation.
- Genotyping.
- Pathogen detection.

Recommendations and Guidelines for qPCR Reaction

Instrument Compatibility

This kit can be used with a variety of real-time instruments, including but not limited to the Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96, Eppendorf, ABI Prism7500/7500 Fast, QuantStudio® 3 System, QuantStudio® 5 System, QuantStudio® 6 Flex System,

QuantStudio® 7 Flex System, ViiA 7 system, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, ABI Prism7000/7300/7700/7900, ABI Step One/Step One Plus. Optimal cycling conditions will vary with different instruments.

Template

Starting material can range from 1 to 100 ng of genomic DNA. If you are using purified cDNA, the amount of template may be as low as 0.1 ng.

ROX Reference Dye

ROX Reference Dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments that are compatible with that option. ROX is supplied at a 50 µM concentration. Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per ml of qPCR Master Mix
Applied Biosystems: ABI 7000, 7300, 7700, 7900HT, StepOne, StepOne Plus	20 µL
Applied Biosystems: 7500, 7500 Fast, Viia7, QuantStudio 3, QuantStudio 5, QuantStudio 6 Flex, QuantStudio 7 Flex. **Stratagene:** Mx3000P, Mx3005P, Mx4000	2 µL
BioRad: iCycler iQ, MyiQ, iQ5, CFX-96, CFX-384. **Eppendorf:** Mastercycler ep realplex. **Roche:** LightCycler 480, LightCycler 2.0.	None

Melting Curve Analysis

Melting curve analysis should always be performed during qPCR to identify the presence of primer dimers and analyze the specificity of the reaction.

Reaction Setup and Conditions

Keep all components, reaction mixes and samples on ice.

General Protocol

Assemble qPCR reactions in a nuclease-free environment. Use of “clean” dedicated pipettes and aerosol resistant barrier tips are recommended.

1. Thaw template DNA and all reagents on ice. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.
2. Prepare the following reaction mixture in a qPCR tube on ice:

Component	Volume	Final Concentration
Template DNA	x µL	1-100 ng
2×SYBR Green qPCR Master Mix	10 µL	1×
Forward Primer (10 µM)	0.5 µL	250 nM
Reverse Primer (10 µM)	0.5 µL	250 nM
Nuclease-free H_2O	to 20 µL	-

   Note: Check the instrument to determine the ROX amount to be added to qPCR Master Mix.
3. Mix thoroughly and carefully by vortexing for 3 -5 seconds. Centrifuge briefly to collect the contents of the tube.
4. Perform qPCR reaction using the recommended thermal cycling conditions outlined below:

Steps	Temperature	Duration	Cycle
Initial Denaturation	95ºC	3 min	1
Denaturation	95ºC	15 sec	40
Annealing/Extension	60ºC	60 sec	
Melting Curve	According to the instrument guidelines		

Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
- qPCR Master Mix component is light sensitive; avoid prolonged exposure to intense light.
- Start reaction as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to qPCR reaction.