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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 Greem qPCR Master Mix	2×1 mL	5×1 mL	-20 °C, avoid repeated freeze-thaw	The product is stable for one year when stored as directed.
ROX Reference Dye (25 μM)	100 μL	200 μL		
Low ROX Reference Dye (2.5 μM)	100 μL	200 μL		

Product Description

ABP SYBR Green qPCR Master Mix is a ready-to-use solution optimized for qPCR and 2-step RT-qPCR. The master mix includes HotStart *Taq* DNA Polymerase and dNTPs in an optimized PCR buffer. Only template and primers need to be added. The SYBR Green I allows for DNA detection and analysis without using sequence-specific probes.

HotStart *Taq* DNA Polymerase in combination with an optimized buffer ensures PCR specificity and sensitivity. 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.

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	1-500 ng
2×SYBR Green qPCR Master Mix	10 µL	1×
ROX Reference Dye (25 µM) or Low ROX Reference Dye (2.5 µM) or No ROX	0.4 µL 0.4 µL -	500 nM 50 nM -
Forward Primer (10 µM)	0.6 µL	300 nM
Reverse Primer (10 µM)	0.6 µL	300 nM
Nuclease-free H_2O	to 20 µL	-

Note: Check Table 1 for final concentration of ROX optimal for your instrument.

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	95^{o}C	5 min	1
Denaturation	95^{o}C	15 sec	40
Annealing/Extension	60^{o}C	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 RT-qPCR reaction.

Table 1. Recommended amounts of ROX Reference Dye for a specific instrument.

Instrument	Volume (20 µL rxn)	ROX Selection
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.4 µL	ROX Reference Dye (25 µM)
Applied Biosystems: 7500, 7500 Fast, Viia7. **Stratagene:** Mx3000P, Mx3005P, Mx4000	0.4 µL	Low ROX Reference Dye (2.5 µM)
BioRad: iCycler iQ, MyiQ, iQ5, CFX-96, CFX-384. **Eppendorf:** Mastercycler ep realplex. **Roche:** LightCycler 480, LightCycler 2.0. **Corbett:** Rotor-Gene 3000, 6000.	None	No ROX