2×TaqProbe qPCR Master Mix

Catalog Number: D027-1, D027-2

Table 1. Kit Components and Storage

Kit Component	D027-1	D027-2	Storage	Stability
2×TaqProbe qPCR Master Mix	4×1.25 mL	8×1.25 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 TaqProbe qPCR Master Mix is a ready-to-use solution optimized for TaqMan probe-based real time PCR analysis of DNA samples. The master mix includes HotStart *Taq* DNA Polymerase, $MgCl_2$, dNTPs and other proprietary buffer components, which have been optimized to deliver superb sensitivity and performance in under one hour.

HotStart *Taq* DNA Polymerase has 5'→3' polymerase activity and 5'→3' exonuclease activity, which is essential for TaqMan probe hydrolysis during qPCR. It does not have 3'→5' exonuclease (proofreading) activity, and produces 3'-dA-tailed amplicons, which are compatible with TA cloning if needed.

TaqProbe qPCR Master Mix is suitable for a variety of applications, including SNP genotyping assays, gene expression analysis, microarray validation, and high-throughput screening.

Special Features

- Specificity: TaqMan probe-based qPCR.
- 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.
- Microarray validation.
- SNP Genotyping.
- High-throughput screening.

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

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×TaqProbe qPCR Master Mix	10 µL	1×
Forward Primer (10 µM)	0.5 µL	250 nM
Reverse Primer (10 µM)	0.5 µL	250 nM
TaqMan Probe (10 µM)	0.4 µL	200 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 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	

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.