Q-PLUS™ Universal Probe qPCR Master Mix (2X)

Ordering Info

TBK0068, 400 reactions

Description

The Q-PLUS™ Universal Probe qPCR Master Mix (2X) is an optimized, ready-to-use kit designed for Real-time PCR (qPCR) with all standard qPCR machines. This Master Mix is provided as a 2× reaction mixture that contains all the necessary components required for real-time PCR, including dNTPs, stabilizers, and enhancers.

The Q-PLUS™ Probe qPCR Master Mix (2X) contains a Hot-Start Taq DNA Polymerase that has been chemically modified. This polymerase is inactive at room temperature, which helps to prevent the extension of non-specifically annealed primers or primer dimers, leading to a higher specificity of DNA amplification.

In addition, this kit includes a tube containing Internal Control (IC) and a tube containing IC primers/Probe (YY-TAMRA). The IC included in the Master Mix serves to verify that the PCR procedure was done correctly.

This kit also includes a separate vial of ROX that can be optionally added to the qPCR reaction Mix. The final concentration of ROX will vary depending on each real-time cycler manufacturer's specification.

Applications

- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis.

Kit Components

Components	TBK0068
Q-PLUS™ Probe qPCR Master Mix (2X)	4 x 1.25 mL
IC template DNA	200 µL
IC Primers/Probe Set	1.1 mL
ROX passive reference dye, 25 µM	200 µL
PCR grade water	4 x 1.5 mL

Q-PLUS™ Probe qPCR Master Mix (2X) (TBZ0220); IC template DNA (TBR0274); IC Primers/Probe Set (TBR0275); ROX passive reference dye (TBR0276); PCR Grade Water (TBB0303)

Storage

Shipped on blue ice. Upon receipt, kit components should be immediately stored at -20°C. Avoid repeated freezing and thawing. Maintain cold when thawed.

IPC Primers/Probe Set must be maintained in the dark. Mix gently and aliquot in different tubes. Store aliquots at -20°C.

Quality Control

- Q-PLUS™ Universal Probe qPCR Master Mix (2X) is free of contaminating DNase and RNase.
- Functionally tested.

Technical support

Help and support are available on our website at https://tiarisbiosciences.com/technical-support/.

Alternatively, you can write an email to support@tiarisbiosciences.com with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions

PROTOCOL

This protocol serves as a guideline for qPCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

1. Thaw all required reagents completely and put them on ice.

2. Determine the number of reactions to be set up (add 1 or 2 reactions to this number to compensate for pipetting error) and prepare the reaction mix (minus the DNA template) by combining the Q-PLUS™ Probe qPCR Master Mix (add ROX as necessary), IC Primers/Probe Set, IC template DNA, Target specific primer/probe mix and Nuclease-Free Water as described below. Vortex briefly to mix. The DNA template is added in Step 4.

Reaction setup (25 µL PCR reaction):

Reaction Components	Volume[1]	Final concentration
Q-PLUS™ Probe qPCR Master Mix (2X)	12.5 µL	1X
25 µM ROX passive reference dye (Optional)[2]	0.5 µl	500 nM/ 50 nM
IC Primers/Probe Set	2.5 µL	-
IC template DNA	0.5 µL	-
Target specific primer/probe mix [3]	X	0.4 µM forward primer 0.4 µM reverse primer 0.2 µM probe
Template DNA	X	gDNA: 20 ng (1 – 100 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
RNase-Free Water	to a final volume of 25µl	

1- *Multiply all numbers according to experimental requirements*
2- *ROX passive reference: ROX is optional for some PCR instruments, and is required as a passive reference dye to normalize small well to well detection differences. Please follow instructions described in the section "ROX reference dye" below for the recommended ROX concentration for your instrument*
3- *The concentrations of primers and probe should be optimized for each primer combination. The recommendation for final primer concentration is 0.4 µM but it can be varied in a range of 0.1-0.8 µM if needed. Optimal results may require a titration of DNA probe concentration between 50 and 800 nM.*

3. Distribute the mix prepared in each PCR tube or well.

4. Add template DNA into each PCR tube or well, according to your experimental plate set up. For negative control use RNase/DNase free water. The final volume in each PCR tube or well is 25µl.

5. Gently mix without creating bubbles (Bubbles interfere with detection of fluorescence).

6. Seal the tubes or optical plate.

7. Program the thermal cycler according to the manufacturer's instructions.

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results:

Process	Cycles	Temperature	Time
Activation[1]	1 x	95 °C	15 min
Denaturation[2]	40 x	95 °C	15-30 sec
Annealing/Extension[3-4]		55-65 °C	30-60 sec

1. It is important to apply 15 minutes 95°C! *Enzyme requires initial activation by incubation at 95°C for 15 min.*

2. Denaturation and Annealing/Extension times can vary between thermocyclers and qPCR master mixes!

3. Choose an appropriate annealing temperature for the primer set used.

4. Combined annealing/extension step with fluorescence data collection.

8. Store the PCR samples at -20°C.

▪ ROX Reference Dye

The passive reference dye ROX is necessary for certain real-time PCR machines as it compensates for non-PCR-related variations in fluorescence detection. The fluorescence emitted from the ROX dye remains constant throughout the real-time PCR process, providing a stable baseline against which PCR-related fluorescent signals can be normalized. As a result, the ROX dye can compensate for any differences in fluorescence detection between wells that may arise from slight variations in reaction volume or differences in well position.

Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per 25 µl reaction	Final ROX concentration
ABI 5700, 7000, 7300, 7000, 7900HT, StepOne™ and StepOnePlus™.	0.5 µL	500 nM (High ROX)
ABI 7500, Stratagene Mx3000P®, Mx3005P™ and Mx4000®.	0.5 µL 10 x diluted* (in water)	50 nM (Low ROX)
BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96 Touch, CFX-384 Touch, Chromo4, MiniOpticon **Qiagen:** Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000 **Eppendorf:** Mastercycler realplex **Illumina:** Eco RealTime PCR System **Cepheid:** SmartCyler **Roche:** LightCycler 480, LightCycler 2.0	Not required	-

* Add 5 µl of ROX Solution to 45 µl of Water, nuclease-free, mix and use 0.5 µl for 25 µl qPCR reaction.