

Q-PLUS™ - One-Step Probe qPCR Master Mix (2x)

Ordering Info

TBK0009, (SAMPLE)
TBK0010, 100 rxns of 20µl
TBK0011, 500 rxns of 20µl

Description

Q-PLUS™ One-Step Probe qPCR Master Mix (2X) allows first-strand cDNA synthesis and subsequent qPCR in a single-tube reaction procedure, reducing the risk of contamination and significantly minimizing hands-on time. The kit includes our Q-PLUS™ Probe qPCR Master Mix, presented as a 2x reaction mixture. This mix incorporates all essential components for real-time PCR, including dNTPs, stabilizers, and enhancers, designed for the efficient amplification and detection of DNA in qPCR based on a wide range of probe-based technologies, including Taqman®, Molecular Beacons® and Scorpion probes®. In addition, a separate RT mix that comprises a balanced mixture of both Reverse transcriptase and Ribonuclease Inhibitor is also provided.

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.

Features

- High Efficiency in multiplex reactions
- High Efficiency in GC/AT-rich templates
- Early Ct values – Rapid extension rate
- Extreme sensitivity – increased limit of detection
- Compatible with fast and standard PCR program

Kit Components

Components	TBK0010	TBK0011
Q-PLUS™ Probe qPCR Master Mix, (2x)	1ml	5x 1ml
ROX reference dye	1 vial	1 vial
RT Mix	100µl	5 x 100µl
RNase-free Water	1ml	5x 1ml

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.

Applications

- One-Step RT-qPCR
- Absolute quantification
- gene copy number determination.
- Gene expression analysis

Technical Assistance

Please refer any technical questions to support@tiarisbiosciences.com

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 realtime 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.

Depending on your equipment, prior to use for the first time, add 18µl (“High ROX”) of the ROX reference dye to the 1ml Q-PLUS™- Probe qPCR Master Mix, (2x) and vortex briefly. If your instrument is “Low ROX” or “No ROX”, then you must use the mastermix as provided, without the inclusion of ROX. Once ROX has been added, the mastermix can be used directly or stored at -20°C for up to 1 year.

PROTOCOL

1. Gently vortex and briefly centrifuge kit components after thawing.
2. Place a tube on ice and add the following components for each 20 µL reaction. Prepare sufficient master mix for the number of reactions. Consider one or two extras:

Components	Volume	Final Concentration
Q-PLUS™ Probe qPCR Master Mix (2x)	10 µL	1x
Forward primer (10µM)	0.8 µl	400nM
Reverse primer (10µM)	0.8 µl	400nM
Probe (10µM)	0.4 µl	200nM
RT Mix	0.1-1 µl	variable
Template RNA (step 5)	*	*
PCR Grade Water, nuclease-free	up 20 µl	
Final Volume	20 µl	

* In case of total RNA 1pg-1µg; in case of mRNA > 0.01pg; in case of viral RNA 10-10⁸ copies.

3. Dispense the master mix into wells of PCR plate.
4. Gently vortex and spin down the samples.
5. Add in each well the RNA sample. Mix well by pipetting.
6. Seal the PCR plate with optical film.

7. Set-up qPCR cycling (if applicable, select fast mode on the instrument):

Suggested thermal cycling conditions:

Process	Cycles	Temperature	Time
cDNA synthesis	1 x	45°C/55°C *	10-20 min
Enzyme Activation	1 x	95 °C	2-5 min
Denaturation		95 °C	5 sec
Annealing/ Extension**	40 x	60-65°C	20-30sec
Melting	1 x	see your instrument guidelines for setup	

* For most applications, cDNA synthesis should be carried out at 45°C. In case of targets with high secondary structure, synthesis may be optimized by carrying out at 55°C

** Select the shortest time possible but not less than 20 sec and do not exceed 30 seconds. Do not use primers with T_m below 60°C