AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

RealQ Plus 2x Master Mix for Probe

Low ROX™

MADE IN DENMARK

Cat. No.: A314402
400 Reactions (25 µl)

A314402

-	RealQ Plus PCR Master Mix for Probe low ROX™
ID No.	**5000810**
Colour code	**Amber**
A314402	4 x 1.25 ml

Key Features

- Ready-to-use optimized 2x master mix
- Suitable for quantification
- Suitable for multiplexing
- High efficiency and sensitivity
- Wide dynamic range
- Hot start capacity for room temperature setup

Introduction

The RealQ Plus 2x Master Mix for Probe is a single-tube 2x reagent including all components necessary to perform probe based real-time DNA amplification. The RealQ Plus 2x Master Mix for Probe is suitable for multiplexing for up to four DNA targets in the same tube, thereby saving PCR consumables, time, workload and valuable DNA. Just add your probes, primers and DNA.

The RealQ Plus 2x Master Mixes promote high specificity and low background by using TEMPase Hot Start DNA Polymerase, a modified Taq DNA polymerase with hot start capabilities.

Detection limit of RealQ Plus for Probe Low ROX™ is approximately 2 copies (~0.007 ng of human gDNA, correlating to 1 diploid genome, with 2 gene copies per diploid genome). Quantification limit is approximately 24 copies (0.08 ng of human gDNA, correlating to 12 diploid genomes, with 2 gene copies per diploid genome).

Real-time PCR is an important tool for SNP and gene expression analysis.

Composition of RealQ Plus 2x Master Mix for Probe, Low ROX™:

- Optimized buffer system including TEMPase Hot Start DNA Polymerase and dNTPs

Quality Control

TEMPase Hot Start DNA Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity. The RealQ Plus 2x Master Mix for Probe Low ROX™ is functionally tested for efficiency and absence of contaminating human genomic DNA.

Recommended Storage and stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.
Option: Store at +4 °C for up to 3 months.

Pre-protocol Considerations

Primers and Probes

The design of primers and probes is critical especially for successful multiplex real-time PCR

- Design primers with similar annealing temperature.
- Analyse primer and probe sequences to avoid primer/probe hairpins, homo- or heterodimers, or any primer/probe complementarity across the targets.
- Optimization of primer and probe concentrations is highly recommended.
- Test assay efficiency by running each assay in singleplex reactions before conducting multiplex qPCR.
- Choose reporter dyes with appropriate excitation wavelengths with little to no overlap in their emission spectra. Check the instrument manual for recommendations.

Amplicon size

Recommended amplicon size is less than 200 bp.

Preventing Template Cross-Contamination

Due to the high sensitivity of quantitative PCR there is a risk of contaminating the reactions with the products of previous runs. To minimize this risk, tubes or plates containing reaction products should not be opened or analysed by gel electrophoresis in the same laboratory area used to set up reactions.

Instrument compatibility

Real-time instruments which require low ROX™ such: Applied Biosystems® 7500, 7500 Fast and ViiA™7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx.

Protocol

Note:

- Prior to the experiment, it is crucial to carefully optimize experimental conditions and to include controls at every stage. See pre-protocol considerations for details.
- Thaw the RealQ Plus 2x Master Mix. Following initial thawing of the master mix, store the unused portion at +4 °C. **Important:** Multiple freeze-thaw cycles should be avoided.

1. Prepare the experimental reaction by adding the components in the order shown in table 1.

2. Gently mix without creating bubbles* (do not vortex).
   * Bubbles interfere with detection of fluorescence.

3. Place the reaction in the instrument and run the appropriate program according to the manufacturer's instructions.

4. Gently mix without creating bubbles* (do not vortex).
   * Bubbles interfere with detection of fluorescence.

5. Place the reaction in the instrument and run the appropriate program according to the manufacturer's instructions.

Table 1. Reaction components

Component	Vol./reaction	Final concentration
RealQ Plus 2x Master Mix	12.5 µl	1x
Forward Primers (10 µM)	0.25 – 2 µl	0.1 – 0.8 µM
Reverse primers (10 µM)	0.25 – 2 µl	0.1 – 0.8 µM
Probes (10 µM)	0.125 – 0.625 µl	0.05 – 0.25 µM
PCR-grade H_2O	X µl	-
Template DNA	X µl	genomic DNA: 20 ng (1 – 100 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume*	25 µl	-

* If using smaller reaction volumes, scale all components proportionally. Reaction volumes < 10 µl is not recommended. Smaller reaction volumes decrease signal intensity.

Three-step PCR Program

Cycles	Duration of cycle	Temperature
1[a]	15 minutes	95 °C
40	15 – 30 seconds[b] 30 seconds[c] 30 seconds	95 °C 55 – 65 °C[d] 72 °C

Two-step PCR Program (recommended)

Cycles	Duration of cycle	Temperature
1[a]	15 minutes	95 °C
40	15 – 30 seconds[b] 60 seconds[c]	95 °C 55 – 65 °C[d]

[a] For activation of the TEMPase hot start enzyme.
[b] Denaturation time is varying between thermocyclers.
[c] Set the qPCR instrument to detect and report fluorescence during the annealing/extension step of each cycle.
[d] Choose an appropriate annealing temperature for the primer set used.

Related Products

Real-time PCR Master Mixes (400 x 25 µl reactions)	Cat. No.
RealQ Plus 2x Master Mix for probe,	
• without ROX™	**A313402**
• with low ROX™	**A314402**
• with high ROX™	**A315402**
RealQ Plus 2x Master Mix Green	
• without ROX™	**A323402**
• with low ROX™	**A324402**
• with high ROX™	**A325402**

ROX and PCR Grade Water	Cat. No.
ROX Internal Reference Dye 200 µM, 3 x 0.2 ml	**A351513**
PCR Grade Water, 6 x 5 ml	**A351513**

For Research Use Only. Not for use in diagnostics procedures.

Other product sizes, combinations and customized solutions are available. Please look at www.ampliqon.com or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

Made in Denmark

Issued 11/2021

Ampliqon A/S, Stenhuggervej 22, DK-5230 Odense M, Denmark. Phone: +45 70201169 Fax: +45 70201179 info@ampliqon.com www.ampliqon.com