



Applications

- Quantitation – Standard curves and comparative
- Presence/absence
- Genomic DNA and cDNA

Key features

- All-in-one optimized master mix, with or without ROX™ reference dye
- High efficiency and specificity
- High stability and reproducibility
- Hot start capacity for setup at room temperature
- Multiplexing (only for Probe)

Introduction

RealQ Plus 2x Master Mixes are easy to use all-in-one master mixes. RealQ Plus 2x Master Mix has undergone an extensive optimization process by the R&D team of Ampliqon. The mix and enzyme is optimized to have high efficiency when working with gDNA and cDNA in order to reach precise and reliable quantitation and presence/absence results.

Quantitative PCR is an important tool for SNP and gene expression analysis. RealQ Plus 2x Master Mixes are ideal for most quantitative PCR applications. The RealQ Plus 2x Master Mixes have been designed for optimal performance on all real-time PCR cyclers.

The RealQ Plus 2x Master Mixes contain the Ampliqon TEMPase Hot Start DNA Polymerase which improves the PCR amplification reaction by decreasing background from non-specific amplification and increasing amplification of the desired products. Furthermore, the TEMPase is inactive at room temperature and thus eliminates the necessity of working on ice during experiment setup. The RealQ Plus 2x Master Mixes contains dNTP's, MgCl₂ and an optimized buffer system necessary to perform optimal DNA amplification. RealQ Plus 2x Master Mixes can successfully amplify and detect a variety of DNA targets such as cDNA, genomic - and plasmid DNA.

High Efficiency and Specificity

To examine the efficiency of the RealQ Plus 2x Master Mix Green, High ROX™, a 4-fold dilution series with gDNA was set up for PAH target. Samples were made in triplicates starting with 80 ng gDNA per well.

The results depicted in figure 1 show high precision and efficiencies close to 100 %.

The identical melting curves show a high specificity of the product and the standard curve shows linear detection range and a high accuracy of the Ampliqon TEMPase Hot Start DNA Polymerase (figure 1).

Stability

Due to the stability of RealQ Plus 2x Master Mix Green High ROX™ and the inactivity of the TEMPase before Hot Start, the mix allows the scientist to preassemble the qPCR reaction, store it, and run it several hours later, when convenient.

A preassembled qPCR plate was incubated in darkness at room temperature for 72 hours and run at the StepOnePlus instrument, from Life Biotechnologies together with a newly mixed reaction setup. The results showed synchronized Ct values with very low standard deviation (figure 2), thus confirming stability of RealQ Plus 2x Master Mix Green High ROX™.

Compatibility

RealQ Plus 2x Master Mixes are compatible with all real-time PCR cyclers. To select the correct RealQ Plus 2x Master Mix for your instrument, please see table 1.

Detection Limit

- 6 copies – RealQ Plus 2x Master Mix Green
- 2 copies – RealQ Plus 2x Master Mix for Probe

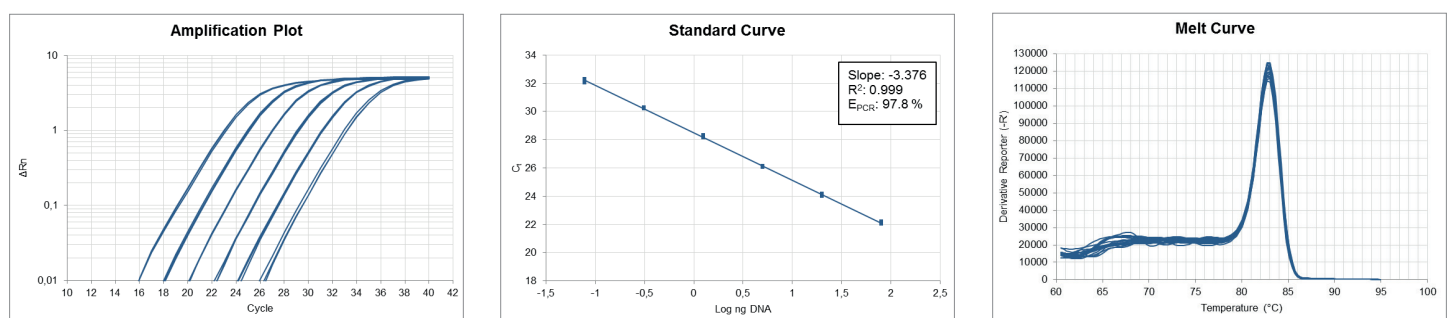


Figure 1. Amplification plot of a 4-fold dilution series for PAH target amplified from human gDNA. Starting amounts of 80 ng gDNA, amplified in triplicates using RealQ Plus 2x Master Mix Green, High ROX™.

REALQ PLUS MASTER MIXES

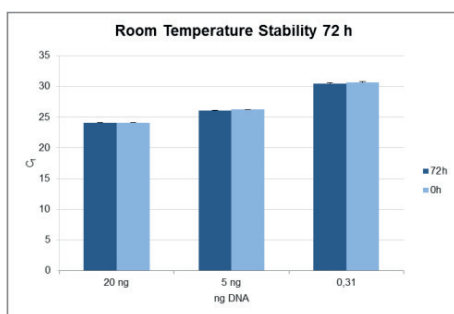
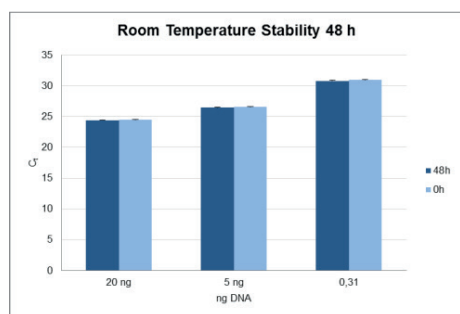
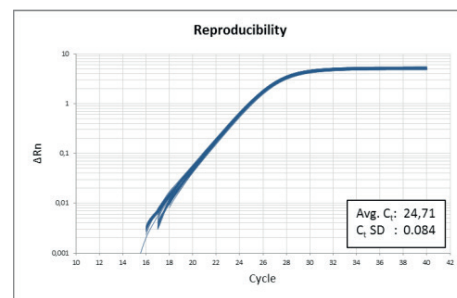
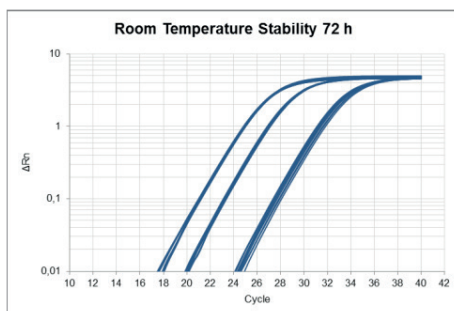
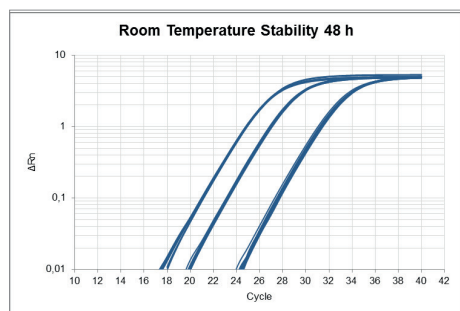


Figure 3. 80 replicates of RealQ Plus 2x Master Mix Green High ROX™ and 20 ng gDNA, show a standard deviation of only 0.084.

Figure 2 (left). Two plates were preassembled for qPCR reaction and incubated in darkness at room temperature for 48 and 72 hours. The results show high stability and complete inactivation of the TEMPase before hot start. It allows the scientist to set up the reaction and run the plate several hours later, when convenient.

Reproducibility and Variability

RealQ Plus 2x Master Mix Green High ROX™ produces reliable and reproducible results. A demonstration of 80 replicates, containing RealQ Plus 2x Master Mix Green High ROX™ and 20 ng gDNA per well, was run on the StepOnePlus instrument from Life Biotechnologies. The results showed identical amplification curves and a standard deviation of 0.084 (figure 3).

Quality Control

In order to ensure high product quality, the TEMPase Hot Start DNA Polymerase is tested for no trace of endonuclease activity, nicking activity, exonuclease activity and priming activity. Furthermore the RealQ Plus 2x Master Mixes are functionally tested for efficiency and absence of contaminating human genomic DNA.

ROX™ level	Instrument compatibility*	400R	4000R
Without ROX™	Bio-Rad CFX96 Touch™, CFX384 Touch™, CFX Connect™, DNA Engine Opticon® 2, Chromo4™, iCycler iQ™ and My iQ™, Roche LightCycler® 480, LightCycler® 1536, LightCycler® Nano, LightCycler® 96 and QuantStudio™ instruments, Thermo Scientific™ PikoReal™, Cepheid SmartCycler®, Bio Molecular Systems Mic qPCR cyler, Qiagen Rotor Gene Q and Rotor Gene 6000.	RealQ Plus 2x Master Mix Green:	
		A323402	A323406
		RealQ Plus 2x Master Mix for Probe:	
Low ROX™	Applied Biosystems® 7500, 7500 Fast and ViA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx.	RealQ Plus 2x Master Mix Green:	
		A324402	A324406
		RealQ Plus 2x Master Mix for Probe:	
High ROX™	Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900 HT, StepOne™ and StepOnePlus™.	RealQ Plus 2x Master Mix Green:	
		A325402	A325406
		RealQ Plus 2x Master Mix for Probe:	
		A315402	A315406

Table 1. Instrument compatibility. Use this table to select the correct level of ROX™ for your RealQ Plus Master Mix in order to match the detection technology of your real-time PCR cyler.

	Size Reaction size: 25 µl	Content	Cat #
RealQ Plus 2x Master Mix Probe High ROX™	400 Reactions	4 x 1.25 ml	A315402
	4000 Reactions	40 x 1.25 ml	A315406
RealQ Plus 2x Master Mix Probe Low ROX™	400 Reactions	4 x 1.25 ml	A314402
	4000 Reactions	40 x 1.25 ml	A314406
RealQ Plus 2x Master Mix Probe Without ROX™	400 Reactions	4 x 1.25 ml	A313402
	4000 Reactions	40 x 1.25 ml	A313406
RealQ Plus 2x Master Mix Green High ROX™	400 Reactions	4 x 1.25 ml	A325402
	4000 Reactions	40 x 1.25 ml	A325406
RealQ Plus 2x Master Mix Green Low ROX™	400 Reactions	4 x 1.25 ml	A324402
	4000 Reactions	40 x 1.25 ml	A324406
RealQ Plus 2x Master Mix Green Without ROX™	400 Reactions	4 x 1.25 ml	A323402
	4000 Reactions	40 x 1.25 ml	A323406