REALQ PLUS MASTER MIXES

AMPLIQON
PCR ENZYMES & REAGENTS

Features

- All-in-one optimized 2x master mixes
- High efficiency and specificity
- High stability and reproducibility
- Hot start capacity for setup at room temperature
- Multiplexing (only RealQ Plus for Probe)

Introduction

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

Quantitative PCR is an important tool for SNP, gene expression analysis and quantification of specific genes. 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, $MgCl_2$ and an optimized buffer system necessary to perform optimal qPCR amplifications. 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, 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.

Applications

- Quantitation – Standard curves and comparative
- SNP and gene expression analysis
- Presence/absence
- Genomic DNA and cDNA

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 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.

The stability of the RealQ Plus 2x Master Mix Green was examined. A preassembled qPCR plate was incubated in darkness at room temperature for 48 hours and 72 hours and run at the StepOnePlus instrument, from Life Biotechnologies together with a freshly mixed reaction setup. The results showed synchronized Ct values with very low standard deviation (figure 2), thus confirming long term stability of RealQ Plus 2x Master Mix Green at room temperature.

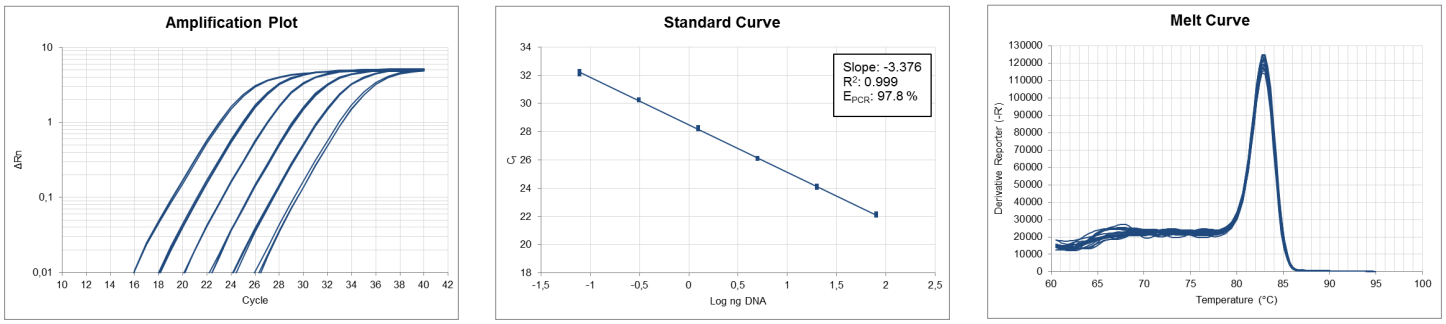

Figure 1. Amplification plot of a 4-fold dilution series for PAH target amplified from human gDNA. Starting amounts of 80 ng gDNA, amplified in triplicates using RealQ Plus 2x Master Mix Green, High ROX™.

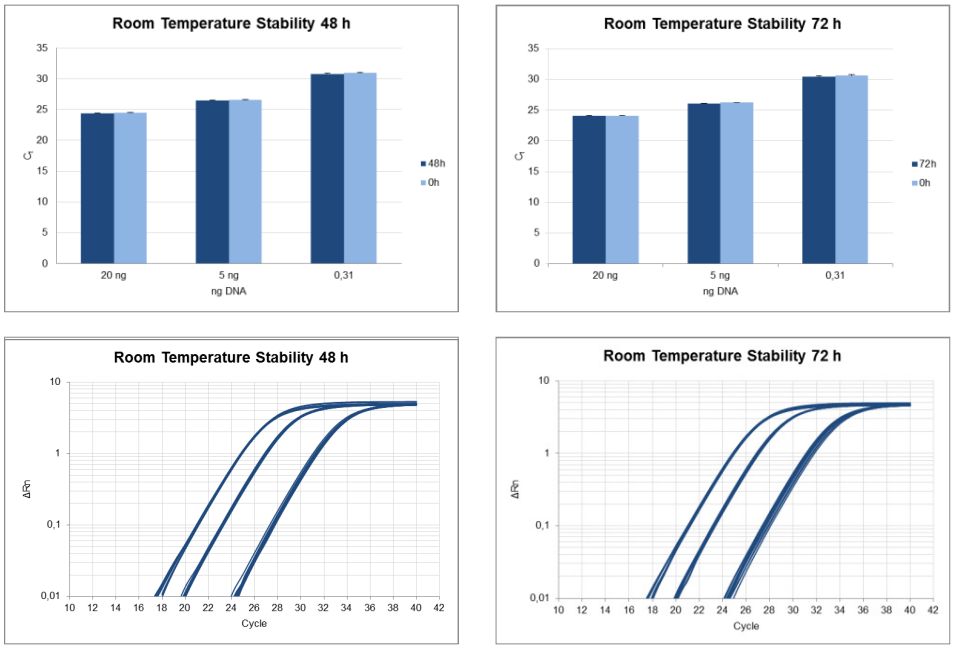

Figure 2 (left)**.** Two plates were preassembled for qPCR reaction and incubated in darkness at room temperature for 48 and 72 hours. The results show high stability and complete inactivation of the TEMPase before hot start. It allows the scientist to set up the reaction and run the plate several hours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

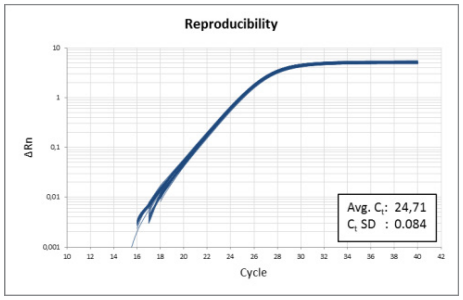

Figure 3. 80 replicates of RealQ Plus 2x Master Mix Green High ROX™ and 20 ng gDNA, show a standard deviation of only 0.084.

Compatibility

RealQ Plus 2x master mixes are compatible with all real-time PCR cyclers. To select the correct concentration of ROX internal reference dye 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

Reproducibility and Variability

RealQ Plus 2x Master Mix Green produces reliable and reproducible results. A demonstration of 80 replicates, containing RealQ Plus 2x Master Mix Green 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).

Multiplexing

The RealQ Plus 2x Master Mix for Probe allows to analyze up to four DNA targets in the same PCR tube. To examine the multiplexing capacity of the RealQ Plus 2x Master Mix for Probe, a serial dilution of four different human gDNA targets were evaluated.

Data showing linear amplification plots of a set of four 5-fold serial dilutions of human gDNA ranging from 40 – 0.32 ng per PCR reaction. Data represents triplicates for each DNA dilution. Cycling was performed on a LightCycler® 96 SW 1.1 with the following protocol: 95°C, 15 min; followed by 50 cycles of 95°C, 15 s; 60°C, 60 s (figure 4 and 5). There is only a minor shift in Cq values for singleplexing vs multingplexing (figure 5).

Quality Control

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

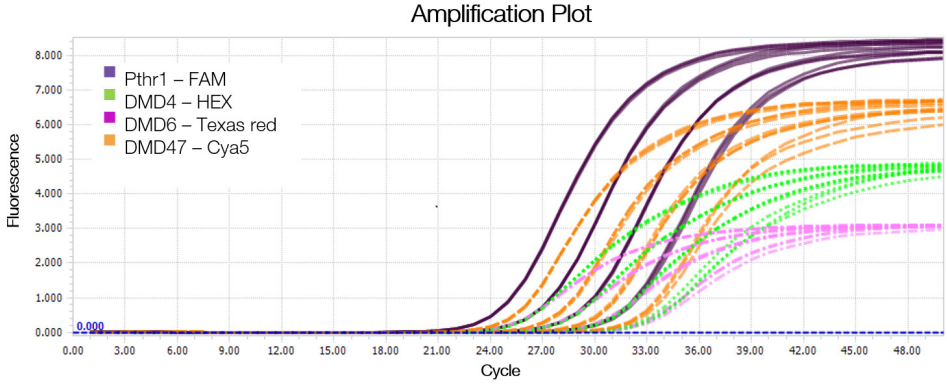

Figure 4. Quadruplex real-time PCR reactions. The Pthr1 (FAM), DMD4 (HEX), DMD6 (Texas red) and DMD47 (Cy5) assays are shown in purple, green, pink and orange respectively.

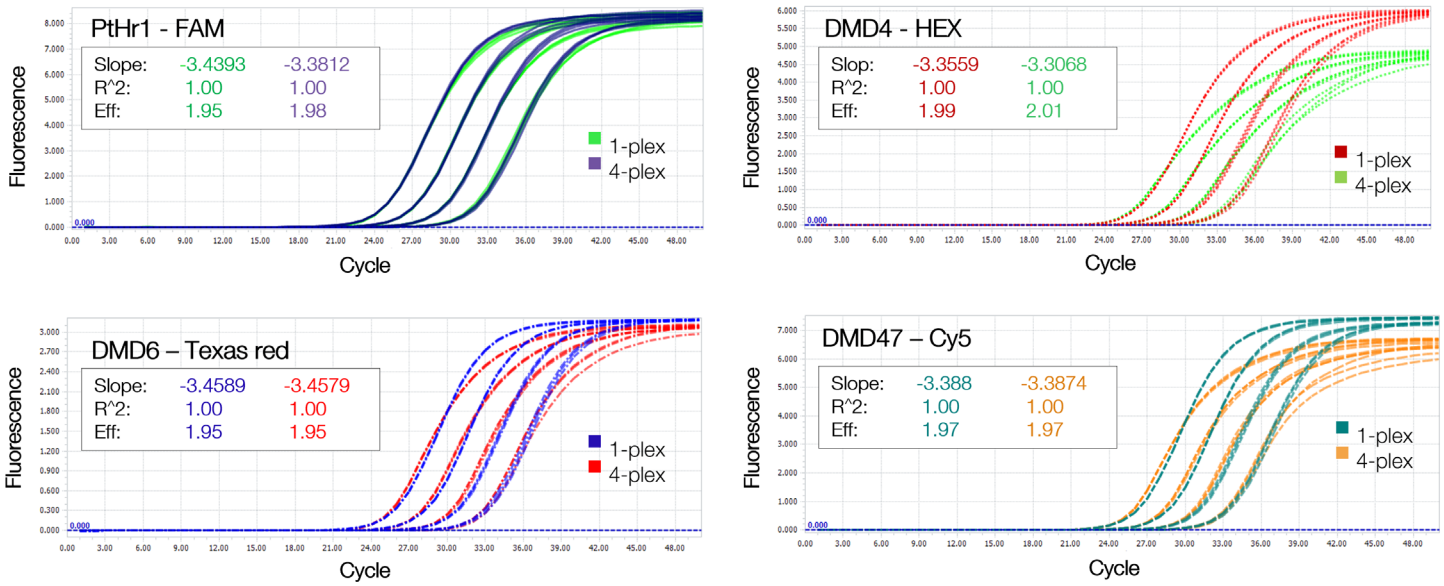

Figure 5. High efficiency, high sensitivity multiplex real-time PCR results with RealQ Plus for Probe. Serial dilution of four different human gDNA targets were amplified either a single-plex real-time PCR, or a 4-target multiplexed real-time PCR.

ROX™ level	Instrument compatibility	400 R	4000 R
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 cycler, Qiagen Rotor Gene Q, Rotor Gene 6000, MyGo Mini and MyGo Pro.	RealQ Plus 2x Master Mix Green:	
		A323402	A323406
		RealQ Plus 2x Master Mix for Probe:	
		A313402	A313406
Low ROX™	Applied Biosystems® 7500, 7500 Fast and ViiA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx.	RealQ Plus 2x Master Mix Green:	
		A324402	A324406
		RealQ Plus 2x Master Mix for Probe:	
		A314402	A314406
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 2x Master Mix in order to match the detection technology of your real-time PCR cycler.

REALQ PLUS MASTER MIXES

Ordering information

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

PCR ENZYMES MADE IN DENMARK

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