AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

RealQ Plus 2x Master Mix Green

Low ROX™

MADE IN **DENMARK**

Cat. No.: A324402

A324402

-	RealQ Plus Master Mix Green Low ROX™
ID No.	**5000840**
Colour code	**Amber**
A324402	1.25 ml

Introduction

The RealQ Plus 2x Master Mix Green with low ROX™ is a single-tube 2x reagent including all components necessary to perform real-time DNA amplification for DNA-binding dye based PCR. Just add your primers and DNA. The ROX™ internal reference dye level is optimized for real-time instruments that require low ROX™ as internal reference dye. See *instrument capability*.

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

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

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

- TEMPase Hot Start DNA Polymerase
- Optimized buffer system including dNTPs, fluorescent dye and ROX™ reference dye

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.

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 Green with low ROX™ is functionally tested for efficiency and absence of contaminating human genomic DNA.

Pre-protocol Considerations

ROX™ Reference Dye

ROX™ is used as passive reference dye to compensate for non-PCR related variations in the fluorescence. The ROX™ fluorescence does not change during the course of the PCR reaction nor does it influence the PCR reaction. It provides a stable baseline to which samples are normalized.

PCR Primers

It is important - especially in fluorescent DNA dye based quantitative PCR applications - to minimize the formation of non-specific amplification products. Particularly at low target concentration it is important to use the lowest possible primer concentration without compromising the efficiency of the PCR. The optimal concentration of primer pairs is the lowest concentration that results in the lowest C_q and an adequate fluorescence for a given target concentration with minimal or no formation of primer-dimers. The optimal concentrations of upstream and downstream primers are not always of equal molarity. Optimal concentrations of primers are in the range of 100 nM to 800 nM.

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. Solutions containing Green DNA dye should be protected from light whenever possible.

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

Table 1. Reaction components (reaction mix and template DNA)

Component	Vol./reaction*	Final concentration*
RealQ Plus 2x Master Mix	12.5 µl	1x
Primer A (10 µM)	0.5 µl (0.25 – 2 µl)	0.2 µM (0.1 – 0.8 µM)**
Primer B (10 µM)	0.5 µl (0.25 – 2 µl)	0.2 µM (0.1 – 0.8 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	-

* Suggested starting conditions; theoretically used conditions in brackets
** Optimization of primer concentrations is highly recommended.
*** If using smaller reaction volumes, scale all components proportionally. Reaction volumes < 10 µl is not recommended. Smaller reaction volumes decrease signal intensity.

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.

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

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

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