AMPLIQON
PCR ENZYMES & REAGENTS

RealQ Plus 2x Master Mix Green

High ROX™

Cat. No.: A325402

A325402

Cat. No.	Reactions (25 µl)	RealQ Plus Master Mix Green high ROX™
ID No. Colour code	-	5000830 Amber
A325402	400	4 x 1.25 ml

Key Features

- All-in-one optimized master mix, including green dye and ROX™ reference dye
- High sensitivity
- High efficiency and high specificity
- Wide dynamic range
- High reproducibility
- Hot Start capacity for room temperature setup

Detection limit: approximately 1 copy.

Quantitation limit: approximately 24 copies
(~0.08 ng of human gDNA, correlating to 12 diploid genomes, with 2 gene copy per diploid genome)

Compatibility: Real-time instruments that require high ROX™ as internal reference dye e.g. StepOne and StepOnePlus from Life Technologies.

Introduction

Quantitative PCR is an important tool for SNP and gene expression analysis. Two general fluorescent chemistries exist for quantitative detection of gene transcripts: probes (e.g. TaqMan®, Scorpions™ Probes, molecular beacons) and DNA-binding fluorescent dyes (e.g. ethidium bromide, SYBR® Green, EvaGreen®, PicoGreen®). Ampliqon offers the RealQ Plus 2x Master Mix in two formulations: for probe or with DNA-binding fluorescent dye, making them ideal for most quantitative PCR applications.

The RealQ Plus 2x Master Mixes are available with high, low or without ROX™ for optimal performance on most of the commonly used real-time PCR instruments. 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.

The RealQ Plus 2x Master Mix Green with high ROX™ is a single-tube 2x reagent including all components necessary to perform DNA-binding dye based real-time DNA amplification. Just add primers and DNA. ROX™ internal reference dye level is optimized for the popular StepOne and StepOnePlus instruments from Life Technologies.

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

- TEMPase Hot Start DNA Polymerase
- Optimized buffer system including dNTPs, green 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 DNA Polymerase is tested for contaminating activities, with no trace of endonuclease activity, nicking activity, exonuclease activity or priming activity. The RealQ Plus 2x Master Mix Green with high 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. The RealQ Plus 2x Master Mix Green with high ROX™ is optimized to be used with StepOne and SteponePlus instruments from Life Technologies.

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_t 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 50 nM to 600 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 analyzed by gel electrophoresis in the same laboratory area used to set up reactions.

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 2.

Table 2. 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 – 2.5 µl)	0.1 µM (0.05 – 0.5 µM)**
Primer B (10 µM)	0.5 µl (0.25 – 2.5 µl)	0.1 µM (0.05 – 0.5 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.

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 – 60 °C[d] 72 °C

Two-step PCR Program

Cycles	Duration of cycle	Temperature
1[a]	15 minutes	95 °C
25 - 35	15 – 30 seconds[b] 60 seconds[c]	95 °C 55 – 60 °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.

Accessories

Reagents	Cat. No.
25mM $MgCl_2$, 3 × 1.5 ml	**A308103**
50x Glass Blocking agent, 3 x 200 µl	**A351413**
ROX™ internal reference dye, 3 x 200 µl	**A351513**

Related Products

Taq Polymerase (500 units) *	Cat. No.
Taq DNA Polymerase 5 U/µl	**A110003**
• with 10x Ammonium Buffer	**A111103**
• 5x PCR Buffer RED	**A111803**
Taq DNA Polymerase 5 U/µl, RED	**A200003**
• with 10x Ammonium Buffer	**A201103**
Taq DNA Polymerase 5 U/µl, glycerol free	**A100003**
• with 10x Ammonium Buffer	**A101103**

Hot Start Polymerase (500 units) *	Cat. No.
TEMPase Hot Start DNA Polymerase, 5 U/µl	**A220003**
• with 10x Ammonium Buffer	**A221103**
• 5x PCR Buffer RED	**A221803**
TEMPase Hot Start DNA Polymerase, glycerol free 5 U/µl	**A240003**
• with 10x Ammonium Buffer	**A241103**

High Fidelity - Proof reading (500 units) **	Cat. No.
AccuPOL DNA Polymerase 2.5 U/µl	**A210003**
• with 10x Ammonium Buffer	**A211103**

*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). **AccuPOL only available in kits with Ammonium Buffer. All kits include extra 25 mM $MgCl_2$.

Buffers for DNA polymerases *	Cat. No.
10x Ammonium Buffer, 3 x 1.5 ml	**A301103**
10x Standard Buffer, 3 x 1.5 ml	**A302103**
10x Combination Buffer, 3 x 1.5 ml	**A303103**
5x PCR Buffer RED, 6 x 1,5 ml **	**A301810**

*Ammonium Buffer, Standard Buffer and Combination Buffer are also available as Mg^{2+} free buffers, detergent free buffers and Mg^{2+} and detergent free buffers. **For direct gel loading and visualisation.

Taq Master Mixes (500 x 50 µl reactions) *	Cat. No.
2x Master Mix, 1.5 mM $MgCl_2$ final concentration	**A140303**
2x Master Mix RED, 1.5 mM $MgCl_2$ final concentration	**A180303**

TEMPase Hot Start Master Mixes (500 x 50 µl reactions) *	Cat. No.
2x Master Mix A**, 1.5 mM $MgCl_2$ final concentration	**A230303**
2x Master Mix A**BLUE, 1.5 mM $MgCl_2$ final concentration	**A290403**

*Master mixes available also in 1.1x variants as well as 2 mM $MgCl_2$ variants, **Mix A is Ammonium Buffer based, also available as Mix C based on Combination Buffer.

Special Master Mixes (500 x 50 µl reactions)	Cat. No.
Multiplex 2x Master Mix, 3 mM $MgCl_2$ final concentration	**A260303**
GC TEMPase 2x Master Mix I – for GC-rich templates	**A331703**
GC TEMPase 2x Master Mix II – for GC-rich templates	**A332703**

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**

Ultrapure dNTPs*	Cat. No.
dNTP Mix 40 mM (2 x 500 µl): 10 mM each dA, dC, dG, dT	**A502004**
dNTP Set, 100 mM each: 250 µl of each dA, dC, dG and dT	**A511104**

*Other concentrations and Single dNTPs are available.

Loading Buffers and Ladders	Cat. No.
5x Loading Buffer Red *, 5 x 1 ml	**A608104**
PCR DNA Ladder **, 100 – 3000 bp, 1 x 0.5 ml	**A610341**

* Also available with Blue, Orange or Cyan. ** Available in different size ranges.

Reagents for *in vitro* laboratory use only.

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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