

RealQ Plus 2x Master Mix for Probe

Low ROX™



A314402

Cat. No.: A314402

Cat. No.	Reactions (25 µl)	RealQ Plus PCR Master Mix for Probe low ROX™
ID No.	-	5000810
Colour code	-	Amber
A314402	400	4 x 1.25 ml

Key Features

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

Detection limit: Approximately 2 copies (~0.007 ng of human gDNA, correlating to 1 diploid genome, with 2 gene copies per diploid genome).

Quantification limit: Approximately 24 copies (0.08 ng of human gDNA, correlating to 12 diploid genomes, with 2 gene copies per diploid genome)

Compatibility: Real-time instruments which require low ROX™ as internal reference dye e.g. the Stratagene MX3005P.

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 and including 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 for Probe with low ROX™ is a single-tube 2x reagent including all components necessary to perform probe based real-time DNA amplification. You just need to add your primers, probe and DNA. The ROX™ internal reference dye level is optimized for real-time instruments that require low ROX™ as internal reference dye e.g. the Stratagene Mx3005P.

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

- TEMPase Hot Start DNA Polymerase
- Optimized buffer system including dNTPs 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 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. The RealQ Plus 2x Master Mix with low ROX™ is optimized to be used with real-time instruments which require low ROX™ as internal reference dye e.g. Stratagene MX3005P.

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 analysed 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.

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)	1 µl (0.5 – 5 µl)	0.4 µM (0.1 – 1.0 µM)**
Primer B (10 µM)	1 µl (0.5 – 5 µl)	0.4 µM (0.1 – 1.0 µM)**
Probe (10 µM)	0.625 µl (0.125 – 0.625 µl)	0.25 µM (0.05 – 0.625)**
PCR-grade H ₂ O	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 and probe concentrations is highly recommended.

- Gently mix without creating bubbles* (do not vortex).
* Bubbles interfere with detection of fluorescence.
- 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	95 °C
	30 seconds ^c	55 – 60 °C ^d
	30 seconds	72 °C

Two-step PCR Program (recommended)

Cycles	Duration of cycle	Temperature
1 ^a	15 minutes	95 °C
40 - 50	15 – 30 seconds ^b	95 °C
	60 seconds ^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 x 1.5 ml	A308103
50x Glass Blocking agent, 3 x 200 µl	A351413
ROX TM internal reference dye, 3 x 200 µl	A351513

The used Hot Start technology is patented in the following countries: Austria, Finland, France, Germany, Great Britain, Italy, Japan, Spain, Sweden, Switzerland and USA. A Hot Start license for use in research in these countries is included with this product, therefore the notice below.

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Related Products

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

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

High Fidelity - Proof reading (500 units) **	Cat. No.
AccuPOL DNA Polymerase 2.5 U/µl • with 10x Ammonium Buffer	A210003 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 MgCl₂.

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 Mg²⁺ free buffers, detergent free buffers and Mg²⁺ 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 MgCl₂ 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 TM • with low ROX TM • with high ROX TM	A313402 A314402 A315402
RealQ Plus 2x Master Mix Green • without ROX TM • with low ROX TM • with high ROX TM	A323402 A324402 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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