



RealQ Plus One-step RT-PCR Kit



Cat. No.: A703302

A703302

200 Reactions (20 µl)

Content	20x RT Mix	RealQ Plus RT 5x PCR Mix	ROX internal reference dye, 200 µM
ID	5700600	5030100	5700300
Colour code	Yellow	White	Amber
Size	1 x 0.4 ml	1 x 0.8 ml	1 x 0.05 ml

Introduction

RealQ Plus One-step RT-PCR Kit includes RealQ Plus RT 5x PCR Mix, the 20x RT Mix, which is a blend of a thermostable reverse transcriptase and RNase inhibitors, and ROX internal reference dye to ensure user-flexibility and compatibility with all standard real-time PCR cyclers.

RealQ Plus RT 5x PCR Mix promotes high specificity and low background by using TEMPase Hot Start DNA Polymerase, a modified Taq DNA polymerase with hot start capabilities.

Applications

- Detection of viral, low copy RNA templates

Benefits

- 1-step reverse transcription and real-time PCR set-up
- High sensitivity
- Efficient cDNA Synthesis ensured by the thermostable Reverse Transcriptase and the advanced RNase inhibitor blend

Pre-protocol Considerations

Protect RNA from degradation

- Take care to prevent RNA degradation by widely spread RNases.
- Prepare RNA samples in dedicated, but different area from the laboratory area used to set up reactions.
- Use nuclease free labware and gloves.

Check quality of RNA sample

Before cDNA synthesis, check RNA quality on a denaturing agarose gel to ensure good quality.

Amplicon size

Recommended amplicon size is less than 200 bp.

ROX reference dye

ROX serves as an internal reference for normalization of the fluorescent signal when using real-time PCR instruments, which can detect ROX.

Table 1. Recommended final ROX concentrations vs. qPCR cyclers

30 nM ROX: Applied Biosystems® 7500, 7500 Fast and ViiA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx.
300 nM ROX final concentration: Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900 HT, StepOne™ and StepOnePlus™.

*For instruments not listed here, please contact technical support at enzyme@ampliqon.com

If needed, prepare a fresh dilution of ROX internal reference dye. For a final reaction concentration of 30 nM dilute 200 µM ROX 1:100 in PCR grade water. For a final reaction concentration of 300 nM dilute 200 µM ROX 1:10 in PCR grade water. For a final reaction volume of 20 µl add 0.3 µl of the ROX dilution. The diluted ROX reference dye must be kept in a light-protected tube at 4 °C.

Protocol

Note:

- Thaw and keep reagents on ice. Mix well before use.
- Keep your bench clean, wear gloves, use sterile tubes and filter pipette tips.

1. Prepare a 20 µl reaction by adding the components in the order shown in table 1.

Table 1. Reaction components

Component	Vol./reaction	Final concentration
Reverse primer		100-400 nM final conc.
Forward primer		100-400 nM final conc.
Specific Probe		200 nM final conc.
ROX 1:100 *	0.3 µl	30 nM – Low ROX
ROX 1:10 *	0.3 µl	300 nM – High ROX
Total RNA or mRNA Template	X µl	1 pg – 1 µg or > 0.01 pg
RealQ Plus RT 5x MM	4 µl	1x
20x RT Mix	1-2 µl	1-2x**
PCR-grade H ₂ O	Add up to 20 µl	
TOTAL volume	20 µl	

*Optional – depending on applied real-time PCR instrument.

**Increased amounts of RT-Mix (up to 2x) improve Cq values, but primer dimers might appear.

2. Gently mix without creating bubbles (do not vortex).
3. Place the reaction in the instrument and run the RT-PCR Program (See next page).

RT-PCR Program

Cycles	Duration of cycle	Temperature
1	Reverse transcription	15 minutes
1	Initial heating	50 – 55 °C
		95 °C
45	Denaturation	5 – 15 seconds
	Annealing/Elongation	30 – 60 seconds
		95 °C
		55 – 65 °C

Recommended Storage

Long term storage at -20 °C.

Related Products

RealQ Plus One-step RT-PCR Kit (20 µl reactions)	Cat. No.
2000 Reactions	A703305
4000 Reactions	A703306

Real-time PCR Master Mixes (400 x 25 µl reactions)	Cat. No.
RealQ Plus 2x Master Mix for probe, <ul style="list-style-type: none">• without ROX™• with low ROX™• with high ROX™	A313402 A314402 A315402
RealQ Plus 2x Master Mix Green <ul style="list-style-type: none">• without ROX™• with low ROX™• with high ROX™	A323402 A324402 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

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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