

## One-step RT qPCR Kit

Cat. No.: A833399  
20 Reactions (20 µl)

	20x RT Mix	4x RT qPCR Mix	ROX internal reference dye, 200 µM
<b>ID</b>	5700800	5030400	5700300
<b>Colour code</b>	Blue	Clear	Amber
<b>Size</b>	1 x 0.02 ml	1 x 0.1 ml	1 x 0.05 ml

### Introduction

One-step RT qPCR Kit includes 4x RT qPCR Mix, the 20x RT Mix, which is a blend of a thermostable reverse transcriptase, RNase inhibitors, and ROX internal reference dye to ensure user-flexibility and compatibility with all standard real-time PCR cyclers.

4x RT qPCR Mix promotes high specificity and low background due to the Hot Start Taq DNA polymerase.

### Applications

- Detection and quantification of low copy RNA templates

### Benefits

- One-step reverse transcription and real-time PCR setup
- 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 a 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.**

<b>30 nM ROX:</b> Applied Biosystems® 7500, 7500 Fast and ViiA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx.
<b>300 nM ROX final concentration:</b> 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](mailto: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

- 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 2.

**Table 2. Reaction components**

Component	Vol./reaction	Final concentration
Reverse primer (10 µM)	0.4-1.4 µl	200-700 nM final conc.
Forward primer (10 µM)	0.4-1.4 µl	200-700 nM final conc.
Specific Probe (10 µM)	0.2-0.7 µl	100-350 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
4x RT qPCR Mix	5 µl	1x
20x RT Mix	1 µl	1x
PCR-grade H <sub>2</sub> O	Add up to 20 µl	-
<b>TOTAL volume</b>	20 µl	-

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

2. Gently mix without creating bubbles (do not vortex).
3. Place the reaction in the instrument and run the RT qPCR Program.

### RT qPCR Program

Cycles		Duration of cycle	Temperature
1	Reverse transcription	10 minutes	50°C (45 – 55°C)
1	Initial heating	3 minutes	95°C
45	Denaturation	10 seconds	95°C
	Annealing/Elongation	30 seconds	55°C (55 – 65°C)

### Recommended Storage

Long term storage at -20 °C.

Made in Denmark

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