

One-step RT qPCR Kit

Cat. No.: **A833305**
2000 Reactions (20 µl)



	20x RT Mix	4x RT qPCR Mix	ROX internal reference dye, 200 µM
ID	5700800	5030400	5700300
Colour code	Blue	Clear	Amber
Size	10 x 0.2 ml	10 x 1.0 ml	2 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.

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

- 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 ₂ O	Add up to 20 µl	-
TOTAL volume	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

Issued 06/2024