AMPLIQON
PCR ENZYMES & REAGENTS

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One-step RT qPCR Kit

For Probe

MADE IN DENMARK

Cat. No.	Reactions (20 µl)	20x RT Mix ID: 5700800	4x RT qPCR Mix ID: 5030400	ROX internal reference dye ID: 5700300
A833301	100	1 x 0.1 ml	1 x 0.5 ml	1 x 0.05 ml
A833302	200	1 x 0.2 ml	1 x 1.0 ml	1 x 0.05 ml
A833305	2000	10 x 0.2 ml	10 x 1.0 ml	2 x 0.05 ml
A833399	Sample - 20	1 x 0.02 ml	1 x 0.1 ml	1 x 0.05 ml

Introduction

One-step RT qPCR Kit for Probe 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 for different real-time PCR 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 * ROX 1:10 *	0.3 µl 0.3 µl	30 nM – Low ROX 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_2O	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 Annealing/Elongation	10 seconds 30 seconds	95°C 55°C (55 – 65°C)

Recommended Storage

Long term storage at -20 °C.

For Research Use Only. Not for use in diagnostics procedures.

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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