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DATA SHEET

Version: 03
Revision date: 27/03/2023

1. Identification

Product name

qMAXSen™ One-Step Probe RT-qPCR Kit (High Rox™)

500 rxn (of 20 µL)

Cat. No: **E0854**

2. Description

qMAXSen™ One-Step Probe RT-qPCR Kit (4X) (High Rox™) allow efficient cDNA synthesis and qPCR in a single tube. The kit includes a qPCR master mix supplied in a 4X concentration to perform real-time PCR. The qPCR master mix contains all the reagent (except PCR primers and template) needed for running PCR reactions. The mix is compatible with many probe technologies. In addition, a separate RT mix that comprises a balanced mixture of both RTase and RNase Inhibitor is also provided.

RT-PCR is used to amplify double-stranded DNA from single-stranded RNA templates. In the RT step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template. In the first cycle of the PCR step synthesis, Taq DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. During subsequent rounds of cycling the DNA polymerase exponentially amplifies the double stranded DNA template.

3. Composition

Item	Quantity
One-Step Probe RT-qPCR (4X) (High ROX)	5 x 500 µl
RT Mix	5 x 100 µl
RNase-free Water	5 x 1 ml

4. Storage specifications

qMAXSen™ One-step Probe RT-qPCR (4X) (High ROX) is shipped on dry/blue ice. The Kit should be stored at **-20˚C** upon receipt. Avoid repeated freezing and thawing.

5. Features

- Higher specificity, sensitivity, and yield.
- For use on a wide range of probe technologies including Taqman®, Molecular Beacons® and Scorpion® probes.
- Available with ROX™ as reference dye.
- Compatible with most real-time PCR instruments.

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6. Applications

- One step qRT-PCR based on specific probes.
- Detection and quantification of DNA and cDNA targets
- Gene expression
- For use with standard and fast qPCR platforms.
- High throughput applications

7. Recommended Protocol

1. **Thaw Kit components, template DNA, probe, primers, and nuclease-free H2O on ice. Mix each solution well.**

The following protocol is recommended for a 20 µl reaction volume:

2. **Set up the following reaction mixture:**

Component	Volume reaction 20 µL	Final concentration
Forward Primer	X µL	100-400 nM(1)
Reverse Primer	X µL	100-400 nM(1)
Specific Probe	X µL	200 nM(2)
RNA Template	X µL	0.01 pg to 1 µg
One-step Probe RT-qPCR (4X) (High ROX)	5 µL	1X
RT Mix	1 µL	1X(3)
Nuclease-Free Water to a final volume of	20 µL	

(1) Too high primer concentrations result in unspecific amplification and should be avoided.

(2) For optimal performance, use 1 pg – 1 µg Total RNA, or >0.01 pg mRNA.

(3) 1 µl is recommended; 2 µl may increase primer dimers, but improves Ct

3. **Mix reagents completely, and then transfer to a thermocycler.**

4. **Program the appropriate PCR cycling protocol on your real-time PCR instrument.**

Suggested thermal cycling conditions:

Step	Temperature	Duration	Cycles
Reverse Transcription	50°C	10 min	1
Initial activation	95°C	3 min	1
Denaturation	95°C	10 sec	40-45
Annealing/Extension*	60°C- 65°C	30 sec	

* Recommended annealing/extension temperature is primer Tm +2°C. Use gradient PCR to optimize the annealing temperature. Do not use temperatures below 60°C. Do not exceed 30 seconds.

5. **For melt analysis refer to instrument instructions.**

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Notes:

- As with all Real-Time PCR reactions, conditions may need to be optimized. You may be able to adjust your PCR conditions to optimize reaction.
- For efficient amplification under fast cycling conditions use amplicon lengths between 80 bp and 200 bp.
- The shorter the amplicon length the faster the reaction can be cycled. Use maximum 400 bp amplicons.
- Primers should have a predicted melting temperature of around 60˚C.
- For TaqMan® probes choose probe close to 5' primer, avoid terminal guanosine residues.

8. Further information

Product Use Limitations

This product is developed, designed, and sold exclusively only for researchpurposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer

The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. **Canvax Reagents S.L.U**. shall not be held liable for any damage resulting from handling or from contact with the above product.