Data sheet

qMAXsen™ One-Step Probe RT-qPCR Kit(2X)

Catalog Number: E0841 (Low ROX) →100 rxns
Catalog Number: E0842 (High ROX) →100 rxns
Catalog Number: E0853 (Low ROX) → 500 rxns
Catalog Number: E0854 (High ROX) →500 rxns

Introduction

qMAXsen™ One-Step Probe RT-qPCR Kit (2X) allow efficient cDNA synthesis and qPCR in a single tube. The kit includes a qPCR master mix supplied in a 2X concentration to perform real-time PCR. The qPCR master mix contains all the reagent (except PCR primers, probe 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.
Available with the option of ROX™ as the internal passive reference dye. The ROX™ dye provides an internal reference to which the reporter-dye signal can be normalized during data analysis.
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.

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.

Kit contents

Item	100 rxns[1]	500 rxns[2]
One-Step Probe RT-qPCR (2X)	1 mL	5x1mL
RT mix	100 µL	5x100 µL
RNase-free Water	1 mL	5x1mL

[1] *100 rxn of 20 µl;*
[2] *500 rxn of 20 µl;*

Storage:

qMAXsen™ One-Step Probe RT-qPCR Kit(2X) is shipped on dry/blue ice. The Kit should be stored at -20°C upon receipt. Avoid repeated freezing and thawing.

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

Quality Control:

Mix Functionally tested in RT qPCR based on specific probe. Tested for activity, processivity, efficiency, sensitivity and heat activation.

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BASIC PROTOCOL

1. Thaw kit components, template RNA, probe, primers and nuclease-free H_2O 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
RNA template	X µL	0.01 pg to 1 µg (2)
One-Step RT-qPCR (2X)	10 µL	1X
RT mix	1µl	1X(3)
Nuclease-Free Water to 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	Time	Cycles
Reverse Transcription	50°C	10 min	1
Initial activation	95°C	3 min	1
Denaturation	95°C	10 sec	40-45
Annealing/Extension*	60-68°C	30 sec	

* Do not use annealing temperatures below 60°C. Recommendation is primer Tm +2°C or use gradient PCR to optimize the annealing temperature.

5. For melt analysis refer to instrument instructions.

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.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.

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