

# Data sheet

## qMAXSen™ One-Step Green RT-qPCR Kit

Catalog Number: E0831 (Low ROX) →100 rxns

Catalog Number: E0832 (High ROX) →100 rxns

Catalog Number: E0833 (Low ROX) → 500 rxns

Catalog Number: E0834 (High ROX) →500 rxns

### Introduction

Canvax™ One-Step 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 using a proprietary green dye. The qPCR master mix contains all the reagent (except PCR primers and template) needed for running PCR reactions. 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.
- Available with ROX™ as reference dye.
- Compatible with most real-time PCR instruments.

### Kit contents

Item	100 rxns <sup>1</sup>	500 rxns <sup>2</sup>
One-Step RT-qPCR (2X)	1 mL	5x1mL
RT mix	200 µL	5x200 µL
RNase-free Water	1 mL	5x1mL

<sup>1</sup> 100 rxn of 20 µl;

<sup>2</sup> 500 rxn of 20 µl;

### Storage:

Canvax™ One-Step 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:

- Detection and quantification of DNA and cDNA targets
- Gene expression
- For use with standard and fast qPCR platforms
- High throughput applications

### Quality Control:



Functionally tested in RT qPCR on Applied Biosystems StepOne® Real-Time PCR System. Tested for activity, processivity, efficiency, sensitivity and heat activation.

(Continued on reverse side)

Distributed by:

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## BASIC PROTOCOL

1. Thaw kit components, template DNA, primers and nuclease-free H<sub>2</sub>O 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 <sup>(1)</sup>
Reverse Primer	X µL	100-400 nM <sup>(1)</sup>
RNA template	X µL	0.01 pg to 1 µg <sup>(2)</sup>
One-Step RT-qPCR (2X)	10 µL	1X
RT mix	1-2 µl	1X <sup>(3)</sup>
Nuclease-Free Water to final volume of	20 µL	

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

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

<sup>(3)</sup> 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	45-55°C	10 min	1
Initial activation	95°C	2 min	1
Denaturation	95°C	5 s	40
Annealing/Extension*	60-65°C	20-30	

\* Do not exceed 30 seconds. Do not use temperatures below 60°C.

5. For melt analysis refer to instrument instructions.

✓ 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.

## 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](http://www.canvaxbiotech.com) for Material Safety Data Sheet of the product.

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