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DATA SHEET

Version: 03
Revision date: 27/03/2023

1. Identification

Product name **One-Step RT PCR Kit**
100 rxn (of 20µL)

Cat. No **P0062**

2. Description

One-Step RT PCR Kit allow efficient cDNA synthesis and PCR in a single tube. The kit includes a PCR master mix supplied in a 2X concentration to perform standard PCR. The 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.

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 doublestranded DNA template.

3. Composition

Item	Quantity
One-Step PCR Mix (2X)	2 x 1.25 mL
RT Mix	2 x 125 µL
RNase-free Water	2 mL

4. Features

- Higher specificity, sensitivity, and yield.
- Efficient thermostable Reverse Transcriptase and RNase Inhibitor providing high cDNA yields.
- Unique Hot Start Taq DNA Polymerase in a mix with high-quality dNTPs.
- PCR enhancers allowing sensitive low background amplification.

5. Storage specifications

Store all components at -20˚C. Avoid repeated freezing and thawing.

6. Applications

•One-step RT-PCR
•Amplification of GC-rich and complex templates

7. Quality Control

One step RT-PCR using eukaryotic total RNA as a template.

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8. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes 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.

BASIC PROTOCOL

1. **Thaw kit components, template DNA, primers and nuclease-free H_2O on ice. Mix each solution well.**

The following protocol is recommended for a 50 µl reaction volume:

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

Component	Volume reaction 50 µL	Final concentration
One-Step PCR Mix (2X)	25 µL	1X
RT mix	2.5 µl	1X
Forward Primer (10 µM)	2 µL	0.4 µM [1]
Reverse Primer (10 µM)	2 µL	0.4 µM [1]
RNA template	X µL	0.1–1 µg [2]
Nuclease-Free Water to final volume of	50 µL	

[1] A final primer concentration of 0.4 µM for each primer is generally optimal. However, for best results, a primer titration using 0.15–0.5 µM is recommended. [2] For optimal performance, use 0.1–1 µg total RNA or 10–500 ng mRNA.

3. **Mix reagents completely, and then transfer to a thermocycler.**
4. **Program the appropriate PCR cycling protocol on your PCR instrument**

Suggested thermal cycling conditions:

Step	Temperature	Time	Cycles
Reverse Transcription	42˚C	30 min	1
Initial activation	95˚C	3 min	1
Denaturation	95˚C	10 s	35-40
Annealing *	55˚C	30 s	
Extension	72˚C	30-60 s/kb	
Final extension	72˚C	5 min	1
Hold	4˚C	∞	1

* Approximately 5˚C below Tm of primers.

5. **Analyze the amplification product.**

✓ As with all PCR reactions, conditions may need to be optimized. You may be able to adjust your PCR conditions to optimize reaction.