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One-Step RT PCR Kit

Cat. No: XV0062P-F (10 rxns)

Cat. No: XV0062P (100 rxns)

Cat. No: XV0063P (500 rxns)

Introduction

XpressBio's One-Step RT PCR Kit allows for 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 reagents (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 double-stranded DNA template.

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

Application

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

Kit Contents

Item	100 rxns[1]	500 rxns[2]
One-Step PCR Mix (2X)	2 x 1.25 ml	10 x 1.25 ml
RT Mix	2 x 125 µl	10 x 125 µl
RNase-Free Water	2 ml	5 x 2 ml

[1]100 rxn of 50 µl; [2] 500 rxn of 50 µl

Storage

Upon receipt of the kit, immediately store the components at -20 °C. Avoid repeated freezing and thawing.

Quality Control

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

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Protocol

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

1. Thaw kit components, template DNA, primers, and nuclease-free water on ice. Mix each solution well.
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, 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	45-55°C	10-20 min	1
Initial activation	95°C	2 min	1
Denaturation	95°C	10 s	35-40
Annealing *	55°C	10 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.

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