

## Reverse Transcription-Polymerase Chain Reaction (RT-qPCR) Utilizing One Step RT-qPCR Kit

### Introduction

One Step RT-qPCR Kit combines two powerful mixtures: i) Reverse Transcriptase and ii) qPCR Master Mix with SYBR® Green with standard buffer providing improved PCR efficiency, wider dynamic range, superior sensitivity and specificity. The two mixtures require minimal handling during the reaction setup and offer consistent and robust RT-qPCR reactions.

Reverse Transcriptase is a recombinant MMLV reverse transcriptase with reduced RNase H activity and increased thermostability. The kit is highly efficient at producing full-length cDNA from long RNA templates at temperatures between 42-55°C. This kit can be used for gene expression data validation, multiplexing, mutation detection, pathogen and viral detection, and genetically modified organisms (GMO) characterization and Genetic profiling. Its advantages include enhanced efficiency, specificity and sensitivity, compatibility with all real time PCR instruments, superior gene expression results under various cycling conditions, and robust and active cDNA synthesis at temperatures up to 55°C.

GoldBio's qPCR Master Mix with SYBR® Green is a ready-to-use cocktail containing all components except primers and template, for the amplification and detection of DNA in qPCR. The qPCR 2x Master Mix with SYBR® Green contains integrated chemically-modified hot start Taq polymerase, SYBR® Green I fluorescent dye, MgCl<sub>2</sub>, dNTPs and stabilizers. ROX reference dye is not included in the master mix. The amplification step features a high quality hot start Taq DNA Polymerase which offers higher fidelity and better amplification. Here, we present a detailed protocol for reverse transcription of mRNA into cDNA and subsequent quantitative PCR reaction.

### Materials

One Step RT-qPCR Kit (GoldBio Catalog # [R-915](#))

- Reverse Transcriptase
- qPCR 2x Master Mix with SYBR® Green

Not included:

- H<sub>2</sub>O PCR grade
- Reverse and forward primers

## Storage and Handling

- Store One Step RT-qPCR Kit at -20°C.
- These products may be shipped on blue ice and should be stored at -20°C immediately upon arrival. When stored under the recommended conditions and handled correctly, these products should be stable for at least 1 year from the date of receipt.

## Method

1. Place kit components and RNA samples on ice.
2. Mix and then centrifuge briefly to collect contents at the bottom of the tube.
3. Prepare a master mix for each reaction and control requiring Reverse Transcriptase enzyme plus 10% extra to allow for pipetting error according to the following table:

<b>PCR reaction set up:</b>	
<b>Component</b>	<b>Volume</b>
RNA template	Up to 1 µg
Forward primer (5µM)	1.0 µl
Reverse primer (5µM)	1.0 µl
qPCR 2x Master Mix with SYBR® Green	10.0 µl
± Reverse Transcriptase	0.25 µl
H <sub>2</sub> O up to	20.0 µl

4. Mix the reaction mixture thoroughly.
5. Program the thermal cycler according to the manufacturer's instructions.
6. A typical PCR cycling program is outlined in the following table.

<b>PCR cycling conditions:</b>			
<b>Steps</b>	<b>Temperature</b>	<b>Time</b>	<b>Cycles</b>
Initial denaturation	95°C	15 min	1
Denaturation	94°C	30 sec	25-40
Annealing	50-66°C	30 sec	
Extension	72°C	1 min/kb	
Final extension	72°C	5 min	1

**Note:** For the annealing step, anneal at optimal annealing temperature for 30 seconds followed by the minimum time required for data acquisition at 72°C according to instrument guidelines.

7. Place the PCR tubes in the thermal cycler and start the cycling program.
8. Analyze the data according to the manufacturer's protocol.

### Associated Products

- [dNTP Mix \(GoldBio Catalog # D-900\)](#)
- [RT-PCR Kit \(GoldBio Catalog # R-920\)](#)
- [RT-qPCR Kit \(GoldBio Catalog # R-925\)](#)
- [One Step RT-PCR Kit \(GoldBio Catalog # R-910\)](#)
- [First Strand cDNA Synthesis Kit \(GoldBio Catalog # D-925\)](#)
- [Reverse Transcriptase \(GoldBio Catalog # R-900\)](#)
- [qPCR Master Mix with SYBR® Green \(GoldBio Catalog # M-915\)](#)
- [Goof-Proof™ qPCR Master Mix \(GoldBio Catalog # G-700\)](#)
- [Goof-Proof™ qPCR Master Mix with ROX \(GoldBio Catalog # G-705\)](#)
- [Goof-Proof™ Universal Probe Master Mix \(GoldBio Catalog # G-710\)](#)
- [Goof-Proof™ Universal Probe Master Mix with ROX \(GoldBio Catalog # G-715\)](#)
- [EvaGreen® Dye, 2000x in DMSO \(GoldBio Catalog # E-675\)](#)
- [EvaGreen® Dye, 20x in Water \(GoldBio Catalog # E-670\)](#)