BIO BASIC INC.

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# 25K Series Guide for 2X One-Step RT-qPCR MM (Dye)

2X One-Step RT-qPCR MM (Dye)

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# Table of Materials

## Contents for 25K Series for 2X One-Step RT-qPCR MM (Dye)

| Components | Product # | Volume | Rxn |
|---|---|---|---|
| 2X One-Step RT-qPCR MM (Dye) | B639277-0050 | 500uL | 50 |
| 2X One-Step RT-qPCR MM (Dye) | B639277-0100 | 1000uL | 100 |

### Kit Components

| Components | B639277-0050, 50 RXN | B639277-0100, 100 RXN |
|---|---|---|
| 2X One-Step RT-qPCR MM (Dye) | 500uL | 1000uL |
| RT Enzyme Mix | 35uL | 70uL |
| RNase-free dd$H_2O$ | 1mL | 2mL |
| Protocol (Instruction Manual) | 1 copy | 1 copy |

## Intended Use

This product is for scientific research only and must not be used in medical or diagnostic procedures on humans or animals. It cannot be used as food, cosmetics, or household items. Without written permission or authorization, you may not manufacture, offer to sell, sell, import the product, or use any related patents or trademarks associated with the product. If you need additional usage permissions, please contact the manufacturer or visit their website. You must comply with all applicable licensing requirements listed on the product webpage when using this product. It is your responsibility to read, understand, and comply with all restrictive terms of these statements.

## Storage

Store at -20°C upon arrival. Transported frozen. Please refer to the packaging for the expiration date.

## Notes

During the operation of the kit, you should wear a lab coat and latex gloves to avoid contamination of the skin, eyes and clothes, and prevent inhalation into the mouth and nose. If contaminated with skin or eyes, please rinse immediately with clean water or saline, and seek medical help if necessary.

## Quality Control

In accordance with Bio Basic ISO-certified Quality Management System, each lot of the 25K Series for 2X One-Step RT-qPCR MM (Dye) is tested against predetermined specifications to ensure consistent product quality.

## Introduction

The 2X One-Step RT-qPCR MM (Dye) uses RNA as the template, performing RNA→cDNA→qPCR sequentially within a single reaction tube. The detection limit can reach 100pg Total RNA.

This kit features high-quality reverse transcriptase and Taq DNA polymerase, along with a proprietary reaction system developed by our company, which significantly enhances amplification performance and reaction specificity. The one-step format eliminates the need for additional reagent additions or opening tubes mid-reaction, thereby preventing contamination.

This technology has been optimized for dye-based real-time quantitation of target RNA sequences via Excitation (498nm), Emission (522nm) of most real-time instruments. This product supports broad instrument compatibility so no additional ROX is required for normalization.

## Important Considerations (Please read carefully before operation)

Prevent RNase Contamination: RNA samples must be protected from RNase contamination. All equipment used for cDNA synthesis should be treated with a 0.1% DEPC aqueous solution at 37°C for 12 hours, followed by autoclaving at 120°C for 30 minutes to eliminate residual DEPC.

Sterilize Reagents and Equipment: Reagents and equipment for RNA experiments must be sterilized either by dry heat (180°C for 60 minutes) or by the DEPC treatment described above. Any sterile water used must also be DEPC-treated and autoclaved. It is highly recommended to use dedicated equipment, reagents, and sterile water exclusively for RNA work.

Preparation: When setting up multiple reactions simultaneously, keep all components and reactions on an ice bath throughout the setup process.

Primer Restriction: This kit is not compatible with Oligo(dT) or Random Primers for first-strand cDNA synthesis. Use gene-specific primers.

RNA Template Quality: For optimal results, use high-quality, intact RNA template.

## Standard Protocol

The recommended qPCR protocol involves first preparing the reaction mix by combining the 2X One-Step RT-qPCR MM (Dye), target-specific forward and reverse primers, and the DNA template in the appropriate volumes on ice, bringing the total volume to the desired amount with RNase-free water. The qPCR amplification then proceeds with an initial denaturation step, followed by multiple cycles of denaturation and annealing/extension, with fluorescence being measured during each cycle to quantify the target DNA. The annealing/extension temperature and time may need optimization based on primer and probe design and the length of the amplicon.

## 1.0 Reaction Setup

Completely thaw all reagents on ice and mix gently. Prepare the reaction mix in 0.2mL PCR tube on ice as follows (for a 20uL total volume):

| Component | Vol (20uL/rxn) | Final Concentration |
|---|---|---|
| 2X One-Step RT-qPCR MM (Dye) | 10 | 1× |
| Forward Primer (10 uM) | 0.4 | 0.2 uM |
| Reverse Primer (10 uM) | 0.4 | 0.2 uM |
| RT Enzyme Mix | 0.65 | - |
| RNA Template | 0.1-100ng | - |
| RNase-free dd$H_2O$ | Up to 20 | - |
| **Total Volume** | **20** | **-** |

**Note 1:** A final primer concentration of 0.2uM in the reaction system can achieve a good amplification effect. When the reaction performance is relatively poor, the primer concentration can be adjusted within the range of 0.1 – 1.0uM.

**Note 2:** qPCR is extremely sensitive. The accuracy of the amount of template added when establishing the reaction system will have a great impact on the final quantitative results. It is recommended to dilute the template and add it to the reaction system, which can effectively improve the repeatability of the experiment. When the template type is undiluted cDNA stock solution, the volume used should not exceed 1/10 of the total volume of the qPCR reaction.

## 2.0 Amplification Program

## 2.1 Conventional Amplification Program

| Step | Temperature | Time | Cycle |
|---|---|---|---|
| Reverse Transcription | 50℃ | 5 min | 1x |
| Initial Denaturation | 95℃ | 3 min | 1× |
| Denaturation | 95℃ | 10 s | 40× |
| Annealing/Extension | 60℃ | 30 s | |

**Note:** The extension time can be appropriately adjusted based on the length of the target gene.