BIO BASIC INC.

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# 25K Series Guide for 2X HMB Hi-Sensitivity Probe qPCR MM

2X HMB Hi-Sensitivity Probe qPCR MM

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## Contents for 25K Series for 2X HMB Hi-Sensitivity Probe qPCR MM

| Components | Product # | Volume | Rxn |
|---|---|---|---|
| 2X HMB Hi-Sensitivity Probe qPCR MM | B690025-0001 | 1000uL | 40-100 |
| 2X HMB Hi-Sensitivity Probe qPCR MM | B690025-0005 | 5000uL | 200-500 |

## 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.

## System Compatibility

**Applied Biosystems:** 5700, 7000, 7300, 7700, 7900HT Fast, StepOne™, StepOne Plus™, 7500, 7500 Fast, ViiA™7, QuantStudio™ 3 and 5, QuantStudio™ 6,7,12k Flex

**Bio-Rad:** CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4

**Eppendorf:** Mastercycler ep realplex, realplex 2 s;

**Qiagen:** Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000;

**Roche Applied Science:** LightCycler 480, LightCycler 2.0; LightCycler 96;

**Stratagene:** MX3000P™, MX3005P™, MX4000P™;

**Thermo Scientific:** PikoReal Cycler; Cepheid: SmartCycler; Illumina: Eco qPCR;SLAN:SLAN-96S,SLAN-96P.

**Note:** This product is suitable for all qPCR instruments, and there is no need to adjust the concentration of ROX on different instruments.

## Storage

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

## Notes

After thawing, the Master Mix may have some floccules. Leave it at room temperature for a while and mix it upside down to dissolve it. It will not affect the performance of the reagent.

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 HMB Hi-Sensitivity Probe qPCR MM is tested against predetermined specifications to ensure consistent product quality.

## Introduction

2X HMB Hi-Sensitivity Probe qPCR MM is an excellent performance detection product designed for high sensitivity and high specificity. This is a ready-to-use, 2X concentrated master mix optimized for highly sensitive and specific quantitative PCR (qPCR) using fluorescent probes (e.g., TaqMan®, FRET, Molecular Beacons). This premix integrates a new generation of antibody-based hot-start Taq DNA polymerase, and specially adds non-specific PCR amplification inhibitors and amplification efficiency enhancement factors, which significantly improves the amplification specificity and detection sensitivity of low-copy genes. It can accurately quantify target genes in a wide quantitative range and detect single-copy genes. It has the advantages of good repeatability, high sensitivity, and high credibility. This product contains all components except primers, probes, and templates, and is easy to use. At the same time, it is compatible with rapid programs to shorten detection time.

## Key Features

**High Sensitivity:** Enables detection of very low target quantities, potentially down to single-copy genes.

**Probe-Based Detection:** Specifically optimized for use with sequence-specific probes, providing excellent specificity.

**Hot-Start Polymerase:** Minimizes non-specific amplification and primer dimer formation, leading to cleaner and more accurate results.

**Broad Instrument Compatibility:** Compatible with a wide range of qPCR instruments.

## Standard Protocol

The recommended qPCR protocol involves first preparing the reaction mix by combining the 2X HMB Hi-Sensitivity Probe qPCR MM, target-specific forward and reverse primers, the target-specific probe, and the DNA template in the appropriate volumes on ice, bringing the total volume to the desired amount with nuclease-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

Prepare the reaction mix in 0.2mL PCR tube on ice as follows (for a 50uL total volume):

| Component | Vol (50uL/rxn) | Vol (20uL/rxn) | Final Concentration |
|---|---|---|---|
| 2X HMB Hi-Sensitivity Probe qPCR MM | 25 | 10 | 1× |
| Forward Primer (10 uM) | 1 | 0.4 | 0.2 uM |
| Reverse Primer (10 uM) | 1 | 0.4 | 0.2 uM |
| Probe (10 uM) | 0.5 | 0.2 | 0.1 uM |
| Template DNA | 2-8 | 1-4 | - |
| Nuclease-Free Water ($ddH_2O$) | Up to 50 | Up to 20 | - |
| **Total Volume** | **50** | **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. The final probe concentration can be adjusted between 50 – 250nM.

**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 |
|---|---|---|---|
| Initial Denaturation | 95℃ | 5 min | 1× |
| Denaturation | 95℃ | 15 s | 40× |
| Annealing/Extension | 60℃ | 30 s | |

### 2.2 Fast Amplification Program

| Step | Temperature | Time | Cycle |
|---|---|---|---|
| Initial Denaturation | 95℃ | 2 min | 1× |
| Denaturation | 95℃ | 5 s | 40× |
| Annealing/Extension | 60℃ | 15 s | |

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