BIO BASIC INC.

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25K Series Guide for 2X HMB Hi-Sensitivity Probe RT-qPCR MM (UDG plus)

2X HMB Hi-Sensitivity Probe RT-qPCR MM (UDG plus)

Table of Contents

Table of Materials

Contents for 25K Series for 2X HMB Hi-Sensitivity Probe RT-qPCR MM (UDG plus)

Components	Product #	Volume	Rxn
2X HMB Hi-Sensitivity Probe RT-qPCR MM (UDG plus)	B639999-0001	1000uL	100

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.

Quality Control

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

Introduction

The 2x HMB RT-qPCR Probe Detection Kit (UDG plus) utilizes an optimized reaction buffer system to support multiplex quantitative PCR reactions using RNA as a template, achieving reverse transcription and quantitative PCR in a single step and the same reaction tube, significantly simplifying the experimental process. The kit integrates a new generation of antibody-based Hot-Start Taq DNA polymerase, combined with non-specific PCR amplification inhibitors and amplification efficiency enhancers, ensuring good detection repeatability, high sensitivity, and strong reliability.

The built-in UDG enzyme can effectively degrade contaminating uracil-containing DNA, avoiding false positives caused by aerosol contamination. The MasterMix also contains BioBasic's proprietary RT.

The kit includes all components except primers, probes, and template, making it easy to use. It is also compatible with fast PCR programs, greatly shortening the detection time. It is suitable for various applications such as pathogen detection and gene expression analysis, making it an ideal choice for RNA detection.

Key Features

Multiplex Capability: Performance in multiplexing applications has been optimized, with sensitive, linear detection of up to 4 or 8 targets.

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.

UDG Carryover Prevention: Contains Uracil-DNA Glycosylase (UDG) and is designed for use with PCR products containing uracil (achieved by including dUTP in the reaction mix). This system effectively eliminates carryover contamination from previous PCRs.

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 RT-qPCR MM (UDG plus), target-specific forward and reverse primers, the target-specific probe, and the RNA template in the appropriate volumes on ice, bringing the total volume to the desired amount with nuclease-free water.

For carryover prevention, the reaction is then typically incubated at a low temperature (e.g., 37°C) for a short period to allow UDG to degrade any uracil-containing contaminant DNA, followed by a high-temperature incubation (e.g., 95°C) to inactivate the UDG and activate the hot-start polymerase.

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 (20uL/rxn)	Final Concentration
2X HMB Hi-Sensitivity Probe RT-qPCR MM (UDG plus)	10	1×
Forward Primer (10 uM)	0.5	0.25 uM
Reverse Primer (10 uM)	0.5	0.25 uM
Probe (10 uM)	0.5	0.25 uM
Template RNA	1-4	-
Sterilized Water (ddH_2O)	Up to 20	-
Total Volume	**20**	**-**

Note 1: A final primer concentration of 0.2 uM 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 – 1.0 uM. The final probe concentration can be adjusted between 50 – 250 nM.

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

A 50°C for 10 minutes is a combined action: it simultaneously converts the RNA template into cDNA for the current reaction and initiates the degradation of any potential uracil-containing DNA contamination.

2.1 Conventional Amplification Program

Step	Temperature	Time	Cycle
Reverse Transcription/Digestion	50℃	10 min	1x
Initial Denaturation	95℃	5 min	1×
Denaturation	95℃	15 s	40×
Annealing/Extension	60℃	30 s	

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