BIO BASIC INC.

Your Supplier and Manufacturer of Life Science Products and Services

25K Series Guide for TaqProbe 2X qPCR-Multiplex MM

TaqProbe 2X qPCR-Multiplex MM

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Contents for 25K Series for TaqProbe 2X qPCR-Multiplex MM

Components	Product #	Volume	Rxn (20uL)	Rxn (50uL)
TaqProbe 2X qPCR-Multiplex MM	B630005	4x 1.25mL	500	200

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 and protected from light. Transported frozen. Please refer to the packaging for the expiration date. We also recommend avoiding multiple freeze-thaw cycles.

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 TaqProbe 2X qPCR-Multiplex MM is tested against predetermined specifications to ensure consistent product quality.

Introduction

The TaqProbe 2X qPCR-Multiplex MM is specifically designed for probe-based real-time PCR analysis of DNA samples. The formulation is optimized for superb performance in sensitivity, signal-to-noise ratio, and complete elimination of primer dimers. This multiplex formulation supports quantitative amplification and detection of up to four targets simultaneously, demonstrating consistent high performance.

This product contains our proprietary HotStart 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 product supports broad instrument compatibility so no additional ROX is required for normalization.

Applications

- SNP Genotyping assays
- Gene expression analysis (using cDNA template)
- Microarray validation
- High throughput screening (up to 4 targets per rxn)

Standard Protocol

The recommended qPCR protocol involves first preparing the reaction mix by combining the TaqProbe 2X qPCR-Multiplex MM, probes, 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 and enzyme activation 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
TaqProbe 2X qPCR-Multiplex MM	10	1×
Forward Primer (10 uM)	variable	0.1-0.5 uM
Reverse Primer (10 uM)	variable	0.1-0.5 uM
Probe (10 uM)	variable	0.1-0.3 uM
DNA template	<500ng	-
RNase-free ddH_2O	Up to 20	-
Total Volume	**20**	**-**

Note 1: Adjust the volume of primers, probes, and DNA template based on their stock concentrations and desired final concentrations/amounts. Adjust RNase-Free ddH_2O to bring the reaction to the final volume.

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
Enzyme Activation	95℃	30 s	1x
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.

3.0 Recommendations

Aliquot Reagents: To prevent contamination and degradation from repeated freeze-thaw cycles, aliquot reagents into smaller, single-use volumes if possible.
Light Sensitivity: The TaqProbe 2x qPCR-Multiplex MM components are light sensitive. Minimize exposure to light during handling and storage.
Prompt PCR Start: Assemble reaction mixtures on ice and start the PCR run as soon as possible after preparation. Always keep the reaction mixture chilled in an ice box prior to placing it in the thermal cycler.

4.0 Troubleshooting Guide

(A) No Fluorescence Signal at All	
Possible Cause	**Suggestion**
Error in thermal cycler program setup	Verify instrument settings match the recommended protocol.
Missing essential components (primers, probe, template)	Double-check reaction assembly for all components.
Probe not labeled correctly or degraded	Use a fresh, correctly labeled probe. Re-label if necessary.
Essential step missing in cycler protocol (e.g., activation)	Confirm all protocol steps are programmed.
Sample configured as empty/blank in software	Check plate setup/configuration in the instrument software.
(B) Late Increase in Fluorescence Signal / Low Efficiency	
Possible Cause	**Suggestion**
Error in thermal cycler program setup	Verify instrument settings.
Insufficient starting template	Increase template amount if possible. Verify template concentration.
Annealing temperature too high	Optimize annealing temperature using a gradient PCR if available. Decrease by 2°C increments.
Probe not labeled well or partially degraded	Re-label or use a fresh probe.
Insufficient extension time for amplicon size	Increase extension time, especially for longer amplicons.
Primer or probe concentration too low	Increase primer concentration (up to max 900 nM each). 250 nM probe is usually sufficient.
PCR protocol not optimal	Ensure recommended protocol is used. Optimize starting from this protocol if needed.
Pipetting error	Check reaction assembly. Ensure accurate pipetting.
Inhibitors present in the DNA sample	Re-purify DNA.
Primer-dimers from previous run contamination	Perform UNG treatment prior to PCR cycling (if using a UDG-compatible system). Maintain good lab hygiene.
Primer and/or probe design not optimal	Re-check primer/probe design. Ensure sufficient template concentration.
(C) Non-Linear Correlation in Standard Curve	
Possible Cause	**Suggestion**
Template dilution inaccurate	Prepare fresh dilution series carefully, ensuring thorough mixing.
Template amount too high (saturation)	Reduce template amount in higher concentration standards; or increase reaction volume if appropriate.
Template amount too low (stochastic effects)	Increase template amount in lower concentration standards.
Primer-dimers co-amplified at low template conc.	Redesign primers to be more specific. Optimize reaction conditions.