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## **25K Series Guide for 2x Multiplex PCR MM**

2x Multiplex PCR MM



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# Table of Materials

## Contents for 25K Series for 2x Multiplex PCR MM

Components	Product #	Rxn
2x Multiplex PCR MM	B639287-0050	50
2x Multiplex PCR MM	B639287-0100	100

### Kit Components

Component	B639287-0050	B639287-0100
Total Reactions	50	100
2x Multiplex PCR MM	1.5 mL	2x 1.5 mL
25 mM MgCl <sub>2</sub> Solution	0.5 mL	1 mL
HotStart Taq DNA Polymerase (5U/ $\mu$ L)	50uL (250 U)	100uL (500 U)
Sterilized ddH <sub>2</sub> O	1 mL	1 x 2 mL
Protocol Booklet	1	1



## **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  $-20^{\circ}\text{C}$  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 2x Multiplex PCR MM is tested against predetermined specifications to ensure consistent product quality.



## Introduction

The 2xMultiplex PCR Master Mix is a ready-to-use solution containing dNTPs, MgCl<sub>2</sub>, multiplex PCR Buffer, and PCR additives designed to improve amplification efficiency and reduce primer-dimer formation. Users need to add template DNA, a primer mix, the provided HS Taq DNA Polymerase, and water to set up the PCR reaction.

Our proprietary HS Taq DNA Polymerase is a hot-start enzyme, inactive at ambient temperatures, preventing nonspecific binding and primer-dimer formation during setup. It is activated by a 4-minute incubation at 95°C. This allows for convenient reaction setup at room temperature.

The 2x Multiplex PCR Master Mix already provides a final MgCl<sub>2</sub> concentration of 4mM in the reaction, which is optimal for most multiplex PCRs. MgCl<sub>2</sub> optimization by titration is usually not required as primer annealing is only marginally influenced.



## Standard Protocol

### Template DNA:

- Multiplex PCR typically requires more template than standard PCR.
- Start with at least 10 ng of genomic DNA per reaction.
- Start with at least 1 ng of less complex DNA (e.g., plasmid, viral DNA).

### Multiplex Primer Design:

- For best results, all PCR products should be less than 1 kb in length.
- If product sizes differ by >1 kb, the primer concentration for the longer amplicon may need to be increased to compensate for lower amplification efficiency.

**Note:** Before multiplexing, amplify each primer set in a singleplex reaction to confirm specificity and efficiency. This can help empirically determine relative primer concentrations for the multiplex reaction.

### Primer Mix Concentration (Final in Reaction):

- The final concentration of each primer in the multiplex PCR reaction should typically be between 50 nM – 200 nM.
- Some primer sets may amplify less efficiently; increasing their concentration may improve yield.

### Reagent Handling:

- Thaw all reagents on ice.
- Mix each solution gently and briefly centrifuge to collect contents.

### Preparation of 10x Primer Stock (2uM of each primer)

Concentration of primer stock	100uM
Each primer	10uL
TE Buffer	Variable
Total volume	500uL

## 1.0 Reaction Setup

Completely thaw all reagents on ice and mix gently and briefly centrifuge:

Component	Vol (50uL/rxn)	Final Concentration
2x Multiplex PCR MM	25	1x
10x Primer Mix (2 uM/primer)	1.25-5	50-200nM
DNA Template	1-500ng	-
HS Taq DNA Polymerase, 5 U/ul	1	5U
Sterilized ddH <sub>2</sub> O	Up to 25	-
<b>Total Volume</b>	<b>50</b>	<b>-</b>

### 1.1 Centrifuge & Overlay

Place the tube in a centrifuge and spin for 30-60 seconds.

If using a thermal cycler without a heated lid, overlay the PCR mixture with mineral oil.

## 2.0 Conventional Thermocycler Program

Step	Temperature	Time	Cycle
Initial Denaturation	95°C	4 min	1x
Denaturation	95°C	15 s	
Annealing	T <sub>m</sub> – 5°C	30 – 60 s	35-40x
Extension	72°C	60 s/kb	
Final Extension	72°C	10 min	1x
Hold	4°C	Hold	

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



### 3.0 Application Example (10-plex Human Genomic DNA)

**Targets:** 10 different regions of human genomic DNA

**Product lengths:** 783, 707, 641, 585, 507, 438, 337, 274, 224, and 127 bp

Component	Volume/Amount
2x Multiplex PCR Master Mix	12.5uL
10x Primer Mix (2uM each primer, for final 60/80/100nM)	Variable
Human Genomic DNA	100ng
HS Taq DNA Polymerase (5U/ $\mu$ L)	0.5uL
Sterilized ddH <sub>2</sub> O	25uL

Centrifuge tube for 30-60 seconds.

### 3.1 Thermocycler Program

Step	Temperature	Time	Cycle
Initial Denaturation	95°C	4 min	1x
Denaturation	95°C	30 s	35x
Annealing	60°C	30 s	
Extension	72°C	60 s	
Final Extension	72°C	10 min	1x
Hold	4°C	Hold	

### 3.2 Products visualized by 3% agarose gel electrophoresis

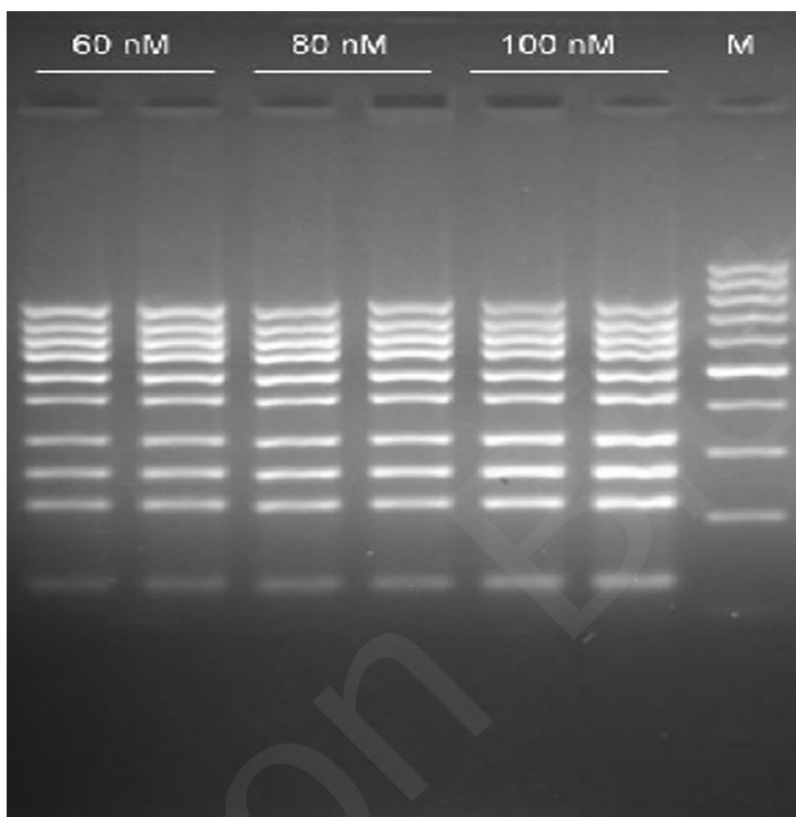


Fig. 1 the agarose gel electrophoresis of the 10-plex PCR products, M: GM343

## 4.0 Troubleshooting Guide

### (A) Little or no PCR product

Possible Cause	Suggestion
Insufficient template DNA	Increase template DNA amount as per protocol guidelines.
Poor template quality	Use purified, high-quality DNA. Evaluate integrity by gel. Store in aliquots to avoid freeze-thaw.
Primer concentration not optimal	Optimize primer concentration in 50 nM increments (range 50-200 nM).
Annealing temperature too high	Decrease annealing temperature in 2-4°C increments.
PCR product is too long	Optimized protocols allow amplification up to 1 kb. For longer targets, ensure sufficient extension time.

### (B) Additional PCR Products are Detectable (Non-Specific Bands)

Possible Cause	Suggestion
Primer design not optimal	Review primer design; check for specificity using BLAST, etc.
Primer degradation	Check concentration and quality of primer solutions. Use fresh primers.
Annealing temperature too low	Increase annealing temperature. Perform a gradient PCR to find optimal annealing temperature.
Too many cycles	Reduce the number of cycles.
Some primers generate >1 specific product	Multiplex primers might bind in close proximity or non-specifically. Re-evaluate primer design or test individual primer pairs.
Too much template	Do not exceed 10 ng (viral/plasmid) or 500 ng (gDNA) per 50 µL reaction.
Amplification of pseudogene sequences	Primers may anneal to pseudogenes. Review primer design to avoid detection of pseudogenes (e.g., by spanning introns or targeting unique regions).