Multiplex PCR Kit

Catalog # PM101

Version 5.1

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Introduction

The Vazyme Multiplex PCR Kit is specially designed for multiplex PCRs, which enables simultaneous amplification of multiple regions of a DNA template or multiple DNA templates. This kit, containing an optimzed hot-start Multiplex DNA Polymerase and 2× Multiplex Buffer, are suitable for almost all multiplex PCR reactions, with high specificity and sensitivity.

Package Information

Components	PM101-01 (50 rxn/50 μl/rxn)	PM101-02 (200 rxn/50 μl/rxn)	PM101-03 (1,000 rxn/50 μl/rxn)
Multiplex DNA Polymerase	50 μl	200 μl	PM101-02 × 5
2× Multiplex Buffer	1.25 ml	4 × 1.25 ml	
5× Multiplex GC Enhancer	700 μl	4 × 700 μl	

Storage

Store at -20℃. Stable for one year.

Recommended PCR System

1. Prepare the 10× Primer Mix.

Mix all the primers into one tube. The final contentration for each primer in 10× Primer Mix is 1 μM.

2. Prepare the reaction solution as follows in a sterile tube:

Nuclease-free water	up to 50 μl
Template [a]	x μl
10× Primer Mix [b]	5 μl
2× Multiplex Buffer	25 μl
5× Multiplex GC Enhancer (**Optional**) [c]	5 - 10 μl
Multiplex DNA Polymerase	1 μl

Notes: a. In a 50 μl PCR system, the recommended template usage is as follows: human genomic DNA, 100 ng; plasmid, 100 pg; cDNA, 1 - 5 μl.

b. The recommended final concentration for each primer is 0.1 μM, which can be modifed between 0.05 - 0.4 μM.

c. The 5× Multiplex GC Enhancer is **ONLY** recommended for templates with complex secondary structure or high GC-content. DON'T use it for nomal multiplex PCRs.

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	5 min [b]	1
Denaturation	95℃	30 sec	35 [e]
Annealing	60℃ [a]	90 sec [c]	
Extension	72℃	60 sec / kb [d]	
Final Extension	72℃	10 min	1

Notes: a. The default anneanling temperature, 60℃, is suitable under most circumstances. However, it can be optimized using gradient PCR if the amplification performance is poor.

b. Per-denaturation at 95℃ for 5 min is necessary to release the activity of Multiplex DNA Polymerase.

c. The annealing time can be extended to 3 min for multiplex PCRs using low-copy templates or with long-fragment amplicons or many amplicons.

d. The extension time can be determined by the longest amplicon. A proper longer extension time is helpful to increase the amplification yield. However, excessive extension may promote non-specific amplification. Therefore, reduce the extension time if specificity is poor.

e. The cycle number can be improved for for multiplex PCRs using low-copy templates. However, excessive PCR cycles may promote non-specific amplification. Therefore, reduce the cycle number if specificity is poor.

Primer Designing Notes

1. It is recommended to use primers with length between 21 - 30 bp, GC-content between 40% - 60%, and annealing temperature is > 68℃.
2. The sizes of amplicons should be < 1500 bp.