2× Phanta™ Max Master Mix

Catalog # P515

Version 5.1

Vazyme biotech co., ltd.

Introduction

Phanta™ Max Super-Fidelity DNA Polymerase is a superior enzyme in this category for robust PCR with extreme fidelity, featuring 53x error rate lower than Taq polymerase and extension rate as fast as 1 sec / kb*. Phanta™ Max Super-Fidelity DNA Polymerase possesses 5´→ 3´polymerase activity,→5´exonuclease activity and will generate blunt-ended products. This product is also capable of amplifying long fragments such as 20 kb genomic DNA and 40 kb λDNA. Our suggested applications include cloning, sequencing, genomic DNA amplification and high throughput PCR, etc.

* The extension rate varies with individual application.

Advantages

Robust: Maximal success with minimal optimization

Extreme Fidelity: 53× error rate lower than Taq

High Yield: Increased product yield using minimal enzyme

Versatile: Used for routine PCR, or with long, difficult templates

Package Information

Components	P515-01	P515-02	P515-03
2× Phanta™ Max Master Mix	1 ml	5 ml	15 ml

All materials should be stored at -20 °C.

Protocol

1. All components should be mixed as recomended below prior to use.

Components	25μl Reaction Volume	50μl Reaction Volume
2× Phanta Max Master Mix	12.5 ul	25 ul
DNA template (100 ng / μl)	variable	variable
Upstream primer (10 μM)	1 ul	2 ul
Downstream primer (10 μM))	1 ul	2 ul
Distilled water (dH_2O)	To 25 ul	To 50 ul
Total reaction volume	25 ul	50 ul

* The recommendation for final enzyme concentration is1 U/ 50 μl, but it can be varied in a range of 0.5 – 2 U/ 50 μl, if needed.

Notes:

a. Add the components into sterile PCR tubes while mixing gently.

b. Place PCR tubes to a PCR cycler.

c. Perform PCR reaction using optimized cycling conditions. Suggested cycling parameters for using Phanta™ Max Super-Fidelity DNA Polymerase are provided below. Analyze PCR amplification products on a 0.7–1.0% (w/ v) agarose gel.

For research use only, not for use in diagnostic procedures.

Segment	Number of cycles	3-step protocol		2-step protocol	
		Temperature °C	Duration	2-step protocol	Duration
Initial Denaturation	1	95	3 min (30 sec)[a]	95	3 min (30 sec)[a]
PCR	25-35[b]	95	15 sec	95	15 sec
		55-65	15 sec	72	15-30 sec/ kb
		72	15-30 sec/ kb		
Final Extension	1	72	5 min	72	5 min
Hold	1	4	∞	4	∞

Notes:

a. This mix is based on a hot-start DNA polymerase, the pre-denaturation activation condition should be set to 95 °C for 3 minutes (for genomic DNA and cDNA) or 30sec (for plasmid DNA and virus DNA) to thoroughly activate the enzyme.

b. Optimized cycling parameters may not necessarily be transferable between thermal cyclers. Consult the instrument manufacturer's recommendations if further optimization of cycling parameters is required.

c. The annealing temperature set up should be based on the Tm of the primers.

d. For primers with annealing temperatures ≥ 72°C, a 2-step protocol is recommended.

Trouble Shooting

No product at all or low yield

1. High quality or purified DNA templates are preferred to enhance the success of PCR.
2. Repeat and make sure that there are no pipetting errors.
3. Use fresh high quality dNTPs.
4. Do not use dNTP mix or primers that contain dUTP or dITP.
5. Sample concentration may be too low. Use more templates.
6. Template DNA may be damaged. Use carefully purified template and make sure template is not fragmented.
7. Increase extension time.
8. Increase the number of cycles.
9. Optimize annealing temperature
10. Optimize enzyme concentration.
11. Optimize the denaturation time.
12. Check the purity and concentration of the primers.
13. Check primer design.

Non-specific products - High molecular weight smears

1. Decrease enzyme concentration.
2. Decrease extension time.
3. Reduce the total number of cycles.
4. increase annealing temperature or try 2-step protocol.
5. Vary denaturation temperature.
6. Decrease primer concentration.

Non-specific products - Low molecular weight discrete bands

1. Increase annealing temperature.
2. Decrease extension time.
3. Decrease enzyme concentration.
4. Titrate template amount.
5. Decrease primer concentration

Limited Product Warranty

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Vazyme.com. We shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

MSDS

Material safety data sheet (MSDS) is available at www.vazyme.com.