



## HotStart PCR Master Mix (2x dye)

### Kit Contents

Components	BS9291 5 x 1 ml
HotStart Master Mix (2x, green dye)	5 x 1 ml
Protocol	1

### Storage and stability

Transportation at 4°C. Store at -20°C.  
Avoid repeated freeze-thaw cycles.

### Introduction

HotStart PCR Master Mix (2X, dye) is a ready-to-use solution containing HotStart Taq DNA polymerase, dNTP, MgCl<sub>2</sub>, PCR buffer, PCR stabilizers, gel loading reagent and dye. PCR products can be loaded onto agarose gel directly. Optimized HotStart Master Mix (2X, dye) can amplify targets up to 5 kb in length from lambda DNA. Users only need to add a template, water and primers to set up a PCR reaction.

### Protocol

- Use the following guidelines to optimize the template concentration:
  - gDNA 1-10 µg/ml
  - plasmid DNA 0.1-1 µg/ml
- Optimizing the Primer Concentration: Primer concentrations in the range of 0.2–0.5 µM work for most PCR amplifications.

- Thaw reagents on ice.

- Prepare a reaction Master Mix using the following protocol:

HotStart Master Mix	25 µl	(1x)
DNA template	1 µl	(0.1-10 ng)
Primer F (10 µM)	2 µl	(0.4 µM)
Primer R (10 µM)	2 µl	(0.4 µM)
Nuclease-free ddH <sub>2</sub> O	20 µl	
<b>Total Volume</b>	<b>50 µl</b>	

- Place the tube in a centrifuge and spin for 30–60 sec.
- Overlay PCR mixture with mineral oil when using a thermal cycler without a heated lid.

Perform most PCR reactions using the following cycling program:

Pre-Duration	94°C	10 min	1 cycle
Duration	94°C	30 sec	30-35 cycles
Anneal	45-68°C	30 sec	
Extend	72°C	1 kb/min	
Post-Extend	72°C	10 min	1 cycle
Hold	4 °C	N.A.	N.A.

## Troubleshooting Guide

Possible Cause	Comment & Suggestions
<i>Little or no product.</i>	
Insufficient template DNA.	Increase template DNA to 0.1–1 µg/50 µl reaction.
Template DNA damaged or degraded.	Always use purified integrated high-quality DNA as templates.
Primer not match.	Review primer design.
Cycling program issue: Annealing temperature too low or too high; Extension time too short; Cycle number not enough.	Check the correct cycling conditions used. Adjust the cycling conditions to optimal. Perform a gradient PCR to determine the optimal annealing temperature if necessary.
Primer concentrations too low.	Use a primer final concentration of 0.2-0.5 µM.
Mg <sup>2+</sup> concentration	Optimize and adjust the

Possible Cause	Comment & Suggestions
not optimal.	concentration of Mg <sup>2+</sup> .
<i>Product is multi-banded.</i>	
Primer design not optimal.	Review primers and check them for specificity.
Primer degradation.	Check the concentration and quality of primer solutions.
Annealing temperature too low.	Increase the annealing temperature. Perform a gradient PCR to find the best annealing temperature.
<i>Product is smeared.</i>	
Too much starting template.	Reduce the amount of template DNA.
Mg <sup>2+</sup> concentration not optimal.	Optimize and adjust the concentration of Mg <sup>2+</sup> .
PCR of long fragments from	Increase Mg <sup>2+</sup> concentration. If the length

Possible Cause	Comment & Suggestions
large genomic DNA.	of the product is > 5kb, please choose long PCR DNA polymerase.
Carry-over contamination.	Purify DNA templates. Preventing carry-over contamination with uracil-DNA glycosylase.



Email  [order@biobasic.com](mailto:order@biobasic.com)  
 Phone  1 (905) 474-4493  
 T. Free  1 (800) 313-7224  
 Fax  1 (905) 474-5794  
 Web  [www.biobasic.com](http://www.biobasic.com)