Taq PCR Master Mix
*(2x, **blue** dye)*

Kit Contents

Components	BS9295 1 ml	BS9296 5 x 1 ml
Taq PCR Master Mix (2x, blue dye)	1 ml	5 x 1 ml
Sterilized ddH2O	1 ml	5 x 1 ml
$MgCl_2$ (25 mM)	0.5 ml	1 ml
Protocol	1	1

QF 24 TV4
CV1 2018

Storage and stability

Transportation at 4°C. Store at -20°C.

Avoid repeated freeze-thaw cycles.

Introduction

Taq PCR Master Mix (2X, blue dye) is a ready-to-use solution containing *Taq* DNA polymerase, dNTP, MgCl2, PCR buffer, PCR stabilizers, gel loading reagent and dye. PCR products can be loaded onto agarose gel directly. Optimized *Taq* PCR Master Mix (2X, blue 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.

The Master Mix provides a final concentration of 1.5 mM $MgCl_2$ in the reaction mix. If a higher Mg^{2+} concentration is desired, please adjust Mg^{2+} concentration accordingly using 25 mM Mg^{2+} solution provided with the Master Mix.

Protocol

1. **Use the following guidelines to optimize the template concentration:**

gDNA	1-10 µg/ml
plasmid DNA	0.1-1 µg/ml

2. **Optimizing the Primer Concentration: Primer concentrations in the range of 0.2–0.5 µM work for most PCR amplifications.**
3. **Thaw reagents on ice.**
4. **Prepare a reaction Master Mix using the following protocol:**

Taq PCR 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 ddH2O	20 µl	
Total Volume	50 µl	

5. **Place the tube in a centrifuge and spin for 30–60 sec.**
6. **Overlay PCR mixture with mineral oil when using a thermal cycler without a heated lid.**
7. **Perform most PCR reactions using the following cycling program:**

Pre-Duration	94°C	4 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
Little or no product.	
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^{2+} concentration not optimal.	Optimize and adjust the concentration of Mg^{2+}.
Product is multi-banded.	
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.
Product is smeared.	
Too much starting template.	Reduce the amount of template DNA.
Mg^{2+} concentration not optimal.	Optimize and adjust the concentration of Mg^{2+}.
PCR of long fragments from large genomic DNA.	Increase Mg^{2+} concentration. If the length of the product is 5 kb, please choose long PCR DNA polymerase.
Carry-over contamination.	Purify DNA templates. Preventing carry-over contamination with uracil-DNA glycosylase.

BIO BASIC INC.

Email order@biobasic.com
Phone 1 (905) 474-4493
T. Free 1 (800) 313-7224
Fax 1 (905) 474-5794
Web www.biobasic.com