2X Hi-Prime *Taq* PCR Master Mix

#G7119 100 Reactions

Store at -20°C

Spin tubes briefly before use

Catalogue Number	Pack Size	☑
G7119	100 Reaction	✓
G7119A	250 Reaction	
G7119B	1000 Reaction	

Introduction

Hi-Prime Taq DNA Polymerase is an optimized combination of **Taq DNA polymerase** and high fidelity DNA polymerases from Pyrococcus species and a best compatible buffer. The 3′→ 5′ exonuclease activity of the high fidelity DNA Polymerase increases the fidelity and robustness in the amplification by Taq DNA Polymerase, even The formulation of this enzyme and buffer has been changed such a way to improve its yield and activity for long PCR. It can be used for PCR of up to 12 kb product.

Guidelines for using 2X Hi-Prime *Taq* PCR Master Mix

The 2X Master Mix contains Hi-PrimeTaq DNA polymerase, Hi-PrimeTaq DNA polymerase reaction buffer, 6mM MgCl2 and 0.4mM of each dNTP.

Package information

G7119 100 Reactions	**2X Hi-PrimeTaq PCR Master Mix (2 x 1.25ml), 10ul Control DNA template, 10ul Control primer Mix**
G7119A 250 Reactions	2X Hi-PrimeTaq PCR Master Mix (5 x 1.25ml), 10ul Control DNA template, 10ul Control primer Mix
G7119B 1000 Reactions	2X Hi-PrimeTaq PCR Master Mix (20 x 1.25 ml), 10ul Control DNA template, 10ul Control primer Mix

Unit definition

One unit incorporates 10nmol of deoxy-ribonucleotide into acid-insoluble product in 30 minutes at 74°C. Unit assay conditions: 25 mM TAPS (pH 9.3), 50 mM KCl, 2 mM MgCl2, 1 mM DTT, 0.2 mM dATP, dCTP, dGTP, dTTP utilizing M13mp18DNA as template..

Enzyme

The amount of enzyme mix will be 1 Unit in 50 µl reactions finally. When cloning fragments amplified with Hi-Prime Taq DNA Polymerase a extra adenine overhang base is incorporated at the 3' end of the amplified PCR product. That A overhang is required to perform the TA cloning.

Table 1. Pipetting instructions (add items in this order).

Component	50 reaction	Final conc.
2x Hi-PrimeTaq PCR Master Mix	25 µl	1X
Forward primer	X µl	0.5µM
Reverse Primer	X µl	0.5µM
Template DNA	X µl	--
H2O	upto 50 µl	--

Table 2. Cycling Instructions

Cycle step	2-step Protocol		Cycles
	Temp.	Time	
Initial Denaturation	94^{0}C	5min	1
Denaturation	94^{0}C	15-30sec	25-35
Annealing	45-63^{0}C	15-60sec	
Extension	68^{0}C	120sec/1kb	
Final extension	68^{0}C	10 min	1
Store	4^{0}C	Hold	

Template

For low complexity DNA (e.g. plasmid, lambda or BAC DNA): 2 pg− 20 ng per 50 µl reaction volume needed to be used. For high complexity genomic DNA, the amount of DNA template should be 20−200 ng per 50 µl reaction volume. If cDNA synthesis reaction mixture is used directly as a source for the template, the volume used should not exceed 10 % of the final PCR reaction volume.

Primers

The recommendation for final primer concentration is 0.5 µM. If required, the primer concentration may be optimized between 0.2−1.0 µM. The results from primer Tm calculations can vary significantly depending on the method used. Always use the Tm calculator and instructions from reputed website to determine the Tm values of primers and optimal annealing temperature. If using a two-step PCR protocol, where both primer annealing and extension occur in a single step at 72°C, the primers should be designed accordingly.

Mg2+ and dNTP

2X Hi-PrimeTaq PCR Mater Mix is optimized to work well for most amplicons. Standard concentration of MgCl2 in final reaction condition is 3mM.

Note

Hi-PrimeTaq DNA Polymerase will not produce 'A' overhang at the 3' end.

DMSO

The recommended reaction conditions for GC-rich templates include 3 % DMSO as a PCR additive, which aids in the denaturing of templates with high GC contents. For further optimization the amount of DMSO should be increased in 2 % increments. In some cases DMSO may also be required for supercoiled plasmids to relax for denaturation. Other PCR additives such as formamide, glycerol, and betaine are also compatible with G9 Taq DNA Polymerase. If high DMSO concentration is used, the annealing temperature must be decreased, as DMSO affects the melting point of the primers. It has been reported that 10 % DMSO decreases annealing temperature by 5.5–6.0°C.

Troubleshooting

Optimize annealing temperature.	**Increase extension time.**
• **Optimize annealing temperature.** • **Repeat and make sure that there are no pipetting errors.**	• Increase the number of cycles.
• **Make sure the cycling protocol was performed as recommended.**	• Titrate DMSO (2−8 %) in the reaction.
• **Use fresh high-quality dNTPs.**	• Denaturation temperature may be too low. Optimal denaturation temperature for most templates is 94-98°C.
• **Do not use dNTP mix or primers that contain dUTP or dNTP.**	• Optimize denaturation time.
• **Titrate template amount. Template DNA may be damaged. Use freshly prepared template.**	• Check the purity and concentration of the primers. • Check primer design.
Non-specific products - High molecular weight smears	
• **Make sure the extension time used was not too long.**	• Titrate template amount.
• **Reduce the total number of cycles.**	• Optimize denaturation temperature.
• **Increase annealing temperature or try 2-step PCR protocol.**	• Decrease primer concentration.
Non-specific products - Low molecular weight discrete bands	
• **Increase annealing temperature**	• Titrate template amount.
• **Make sure the extension time used was not too long.**	• Decrease primer concentration. • Design new primers.

Component	Volume
2X G9 Taq PCR Master Mix	25 µl
Control DNA template	1 µl
Control primer Mix	1 µl
Nuclease-free water	23 µl

Control PCR cycling conditions

Cycling conditions	
Step 1	94˚C – 5 min
Step 2	94˚C – 30s
Step 3	57˚C – 30s
Step 4	72˚C – 4 min
Step 5	72˚C – 10 min
Step 6	4 ˚C – Hold

Repeat Steps 2 to 4 for 40 Cycles. Control PCR will produce a 2Kb product.