2X Hi G9 Taq PCR Master Mix

 # G4804B 100 Reactions

Store at -20°C

Spin tubes briefly before use

Catalogue Number	Pack Size	☑
G4804	1000 Reactions	
G4804A	1000 Reactions	
G4804B	1000 Reactions	✓

Introduction

Codon optimized G9 Taq DNA Polymerase gene of Thermus aquaticus was cloned and purified from E. coli host. The enzyme consists of a single polypeptide with a molecular weight of approximately 94 kDa. Hi-G9 Taq DNA polymerase is heat-stable and will synthesize DNA at elevated temperatures from single-stranded templates in the presence of primer. In Hi-G9 Taq DNA polymerase, G9 DNA polymerase enzyme comes with a newly formulated buffer to improve its yield and activity for long PCR. It can be used for PCR of up to 8 kb product.

Guidelines

The Master Mix contains Hi-G9 Taq DNA polymerase, Hi G9 Taq DNA reaction buffer, 5mM $MgCl_2$, and 0.4mM of each dNTP.

Package information

G4804 (100 Reactions)	Material provided: 2X Hi G9 Taq PCR Master Mix (2 x 1.25 ml), 10ul Control DNA template, 10ul Control primer Mix
G4804A (250 Reactions)	Material provided: 2X Hi G9 Taq PCR Master Mix (5 x 1.25ml), 10ul Control DNA template, 10ul Control primer Mix
G4804B (1000 Reactions)	**Material provided: 2X Hi G9 Taq PCR Master Mix (20 x 1.25ml), 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 optimal amount of enzyme is 1 µl in 50 µl reactions.
*Prime taq DNA polymerase shows 3´→ 5´ exonuclease activity.

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

H2O	To 50µl	
2x PCR Master Mix	25 µl	1X
Forward primer	X µl	0.5µM
Reverse Primer	X µl	0.5µM
Template DNA	X 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	Tm	15-60sec	
Extension	68-72^{0}C	2 min /1kb	
Final extension	68-72^{0}C	5-15min	1

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.

Notes:

- **Use 94°C for denaturation.**
- **The annealing temp depends on the primer.**
- **Use extension at 72°C for upto 6kb amplification and at 68°C for above 6kb amplification.**
- **Use 15–60 s/kb for extension.**
- **Note:** Hi G9 Taq DNA Polymerase produces A overhang at the 3' end

Mg2+ and dNTP

2X Hi G9 Taq PCR Mater Mix is optimized to work well for most amplicons. Standard concentration of MgCl2 in final reaction condition is 2.5 mM.

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

Troubleshooting

Optimize annealing temperature.	**Increase extension time.**
• **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 dITP.**	• 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 Hi G9 Taq PCR Master Mix	25 µl
Control DNA template	1 µl
Control primer Mix	1 µl
Nuclease-free water	23 µl
2X Hi G9 Taq PCR Master Mix	25 µl
Control DNA template	1 µl
Control primer Mix	1 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 25 Cycles. A ~2kb band will be obtained in Control PCR reaction.