GCC BIOTECH®

GSure® Fast Tissue Kit

Prime Taq DNA Polymerase (Recombinant)

100 units (1Units/ul)
Store at -20°C
Spin tubes briefly before use

Catalogue Number	Pack Size	☑
G4798	500 Units	
G4798A	1000 Units	✓
G4799	5000 Units	

Introduction

Prime Taq DNA Polymerase is an optimized combination of Taq DNA polymerase and high fidelity DNA polymerases from Pyrococcus species for use in routine and difficult PCR experiments. The 3´→ 5´ exonuclease activity of the high fidelity DNA Polymerase increases the fidelity and robustness in the amplification by Taq DNA Polymerase, even from very low copy number of template.
Prime Taq DNA polymerase is heat-stable and will synthesize DNA at elevated temperatures from single-stranded templates in the presence of primer. The formulation of this enzyme mix has been optimized such a way to improve its yield and activity for long PCR. It can be used for PCR of up to 10kb product.

Guidelines for using Prime Taq DNA Polymerase

Prime Taq DNA Polymerase is provided with 10X Prime Taq Reaction Buffer. The buffer contains no MgCl2. A separate tube of MgCl2 (25 mM) is provided to maintain the flexibility.

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.

Package information

Component	
Prime Taq polymerase (1 unit/λ)	100 Unit
10X Prime Taq Reactioin Buffer	1 X 500 ul
25 mM $MgCl_2$	1 X 500 ul
Control DNA tempalate	10 µl
Control Primer mix	10 µl

Enzyme

The optimal amount of enzyme is 1 µl in 50 µl reactions.
*Prime taq DNA polymerase shows 3´→ 5´ exonuclease activity.

Table 1. PCR Mix preparation

Component	50 µl Reaction	Final conc.
Template DNA	X µl	<1,000 ng
10X Prime Taq Reaction Buffer	5µl	1X
10 mM dNTPs	1 µl	200µM each
Forward primer#	1 µl	0.2 µM
Reverse primer#	1 µl	0.2 µM
MgCl2#	5 µl	2.5 mM
H2O	To 50 µl	
Prime Taq DNA Polymerase	1 µl	1U

In some template to get optimum result primers conc and $MgCl_2$ conc. needed to be optimized

Protocol

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the components as mentioned in Table 1 for each 50 µL reaction.
3. Mix the samples and spin down as quickly as possible.
4. Perform PCR using recommended thermal cycling conditions (see Table 2). For higher amplification (>4 Kb) extension at 68^{0}C is preferable.

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

Notes:

- **Use 94°C for denaturation.**
- **Use 200 µM of each dNTP. Do not use dUTP.**
- **The annealing temp depends on the PrimerTm**
- **Gently vortex and briefly centrifuge all solutions after hawing..**
- Use 60–120s/kb for extension.

Template

For low complexity DNA (e.g. plasmid, lambda or BAC DNA): 1 pg–10 ng per 25 µl reaction volume or 2 pg– 20 ng per 50 µl reaction volume. For high complexity genomic DNA, the amount of DNA template should be 10–400 ng per 25 µl reaction volume or 20–800 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 0.1−1.0 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

Prime Taq reaction buffer is optimized to work well for most amplicons. Standard concentration of $MgCl_2$ in PCR reaction mixture is 2.5mM but to optimum concentration might vary between 1-5 mM. To keep this flexibility 25 mM $MgCl_2$ provide separately with 10X reaction buffer. High quality dNTPs should be used for optimal performance with Prime Taq DNA Polymerase. The polymerase cannot read dUTP-derivatives or dITP in the template strand so the use of these analogues or primers containing them is not recommended. For optimal results always use 200 µM of each dNTP.

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
Control DNA template	0.5µl
Control primer Mix	0.5 µl
10mM dNTPs	0.5 µl
Prime Taq DNA Polymerase	0.5 µl
Prime Taq Reaction Buffer	2.5 µl
$MgCl_2$	2.5 µl
Nuclease-free water	Upto25 µl

Control PCR set-up

Cycling conditions	
Step 1	94˚C – 5 min
Step 2	94˚C – 30 s
Step 3	57˚C – 30 s
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.