

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 Reaction Buffer	1 X 500 ul
25 mM MgCl ₂	1 X 500 ul
Control DNA template	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
MgCl ₂ #	5 μl	2.5 mM
H ₂ O	To 50 μl	
Prime Taq DNA Polymerase	1 μl	1U

In some template to get optimum result primers conc and MgCl₂ 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°C is preferable.

Table 2. Cycling Instructions

Cycle step	2-step Protocol		Cycles
	Temp.	Time	
Initial Denaturation	94°C	5min	1
Denaturation	94°C	15-30sec	25-35
Annealing	T _m	15-60sec	
Extension	68-72°C	2 min /1kb	
Final extension	68-72°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 PrimerT_m
- Use 60–120s/kb for extension.
- Gently vortex and briefly centrifuge all solutions after hawing..

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 T_m calculations can vary significantly depending on the method used. Always use the T_m calculator and instructions from reputed website to determine the T_m 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.

- Repeat and make sure that there are no pipetting errors.
- Make sure the cycling protocol was performed as recommended.
- Use fresh high-quality dNTPs.
- Do not use dNTP mix or primers that contain dUTP or dITP.
- Titrate template amount. Template DNA may be damaged. Use freshly prepared template.

Increase extension time.

- Increase the number of cycles.
- Titrate DMSO (2–8 %) in the reaction.
- Denaturation temperature may be too low. Optimal denaturation temperature for most templates is 94–98°C.
- Optimize denaturation time.
- 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.
- Increase annealing temperature or try 2-step PCR protocol.
- Optimize denaturation temperature.
- Decrease primer concentration.

Non-specific products - Low molecular weight discrete bands

- Increase annealing temperature
- Make sure the extension time used was not too long.
- Titrate template amount.
- Decrease primer concentration.
- Design new primers.

Component	Volume
Control DNA template	0.5 μl
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.