

Hi Prime Taq DNA Polymerase (Recombinant)

#G7116A 5000 units

Store at -20°C

Spin tubes briefly before use

| Catalogue Number | Pack Size | ☑ |
|------------------|-----------|---|
| G7116 | 1000 Unit | |
| G7116B | 500 Unit | |
| G7116A | 5000 Unit | ✓ |

Introduction

Hi 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 Hi Prime taq DNA polymerase contain a newly formulated 10X buffer to give a better yield and higher sensitivity. Hi 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 and the 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 12kb product.

Guidelines for using Hi-Prime Taq DNA Polymerase

Hi-Prime Taq DNA Polymerase is provided with 10x Hi-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 | G7116B | G7165 | G7116A |
|---|-------------|---------------|----------------|
| Hi Prime Taq DNA polymerase(1.0 unit/λ) | 500 Unit | (2X 500 Unit) | (10X 500 Unit) |
| 10X Hi Prime Taq Reaction Buffer | 1.25 ml X2 | (4X 1.25 ml) | (20X 1.25 ml) |
| 25 mM MgCl ₂ | 2 x 1.25 ml | (4 X 1.25 ml) | (20 X 1.25 ml) |
| Control DNA template | 10 µl | 10 µl | 10 µl |

| | | | |
|--------------------|-------|-------|-------|
| Control Primer mix | 10 µl | 10 µl | 10 µl |
|--------------------|-------|-------|-------|

Enzyme

The optimal amount of enzyme is 1 µl in 50 µl reactions.

*Hi-Prime taq DNA polymerase shows 3'→5' exonuclease activity.

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

| Component | 50 reaction | Final conc. |
|--|-------------|-------------|
| Template DNA | X µl | <1,000 ng |
| 10x Reaction Buffer with MgCl ₂ | 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 | |
| G9 Taq DNA Polymerase | 0.5 µl | 1.25U |

Protocol

- Gently vortex and briefly centrifuge all solutions after thawing.
- Place a thin-walled PCR tube on ice and add the components as mentioned in Table 1 for each 50 µL reaction.

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 |

- Mix the samples and spin down as quickly as possible.
- Perform PCR using recommended thermal cycling conditions (see Table 2). For higher amplification (>4 Kb) extension at 68°C is preferable.

Note

- Use 94°C for Denaturation
- Use 200 µM of each dNTP. Do not use dUTP.
- The annealing temp depends On the Primer T_m
- Gently vortex and briefly centrifuge all solutions after thawing.
- Use 120 s/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 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

G9 Taq reaction buffer is optimized to work well for most amplicons. Standard concentration of MgCl₂ in PCR reaction mixture is 2.5mM but to optimum concentration might vary between 1-5 mM. High quality dNTPs should be used for optimal performance with G9 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.

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 Hi-Prime 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. |
|---|--|
| <ul style="list-style-type: none"> Optimize annealing temperature. | <ul style="list-style-type: none"> Increase the number of cycles. |

- Repeat and make sure that there are no pipetting errors.

- 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 |
|---|------------|
| Control DNA template | 0.5µl |
| Control primer Mix | 0.5 µl |
| 10mM dNTPs | 0.5 µl |
| G9 Taq DNA Polymerase | 0.25 µl |
| 10 X Taq Reaction Buffer with MgCl ₂ | 2.5 µl |
| Nuclease-free water | Upto 25 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.