Gene to Protein Pvt. Ltd.
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800 GENOME, 800 GENETIC

Hot Start Taq DNA Polymerase

Cat # ☐ **PCR352** ☐ **PCR353** ☐ **PCR352-S**
Pack Size: ☐ **500U** ☐ **1000U** ☐ **100U**
Storage: -20°C

Introduction

Gene to Protein Pvt. Ltd's Hot Start DNA Polymerase is an innovative hot-start enzyme. It is the ideal choice for conducting fast PCR reactions with exceptional quality and purity. This high-performance enzyme is suitable for both standard and fast PCR cycling, whether it be for endpoint or real-time assays. With Gene to Protein Pvt. Ltd's Hot Start DNA Polymerase, you can expect high product yields, low background, and primer-dimers or non-specific priming. For optimal results, we recommend using an annealing temperature that is 2°C above the primer Tm. To achieve the best results, you can use a gradient PCR to optimize the annealing temperature.

Features

- Exceptionally pure Taq DNA Polymerase for sensitive PCR applications and high yields.
- No activation steps required, allowing for fast and easy PCR reactions.

Applications

- Fast PCR reactions in both endpoint and real-time analysis.
- Hot-start PCR up to 3 Kb.
- Amplification of low-copy-number targets.
- RT-PCR and TA cloning.

Kit Contents:

Components	PCR352	PCR353	PCR352-S
Hot Start Taq DNA Polymerase	500U (2.5U/µL)- 200 µL	1000U (2.5U/µL)- 400 µL	100U (2.5U/µL)- 40 µL
10X Reaction Buffer	1mL	2mL	500 µL

Product Source

Our Taq DNA Polymerase is sourced from an *E. coli* strain that carries the Taq DNA Polymerase gene from *Thermus aquaticus* YT-11. The 1X Standard Taq Reaction Buffer contains 10 mM Tris-HCl, 50 mM KCl, and 1.5 mM $MgCl_2$ at pH 8.3 @ 25°C.

Storage Buffer: 50 mM Tris-HCl; 100 mM KCl; 1 mM DTT; 0.1 mM EDTA; 50% Glycerol pH 8.0 @ 25°C

Reaction Conditions:

To perform PCR with Gene to Protein Pvt. Ltd's Hot Start DNA Polymerase, use 1X Standard Taq Reaction Buffer, DNA template, primers (not included), 200 µM dNTPs (not included), and 1.5 units of Taq DNA Polymerase in a total reaction volume of 25 µl.

Unit Definition:

One unit of Gene to Protein Pvt. Ltd's Hot Start DNA Polymerase is defined as the amount of enzyme required to catalyze the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74 °C.

Concentration

Gene to protein Pvt. Ltd's Hot start DNA Polymerase has a concentration of 2.5 U/µL.

Storage
Store Gene to protein Pvt. Ltd's Hot start DNA Polymerase at -20°C.

Quality Certifications
• Functionally tested in PCR.
• Undetected bacterial DNA (by PCR).
• Undetectable nucleases activity (endo-, exo-, and ribonucleases).
Guidelines for PCR optimization using Hot start Taq Polymerase:

DNA Template
1. Use high-quality, purified DNA templates.
2. Approximately 10^4 copies are required to detect the amplification in 25-30 PCR cycles.
3. Use higher DNA concentration when few PCR cycles are desired.

Primers
1. Primers should be generally 20-30 bp in size.
2. The GC content should be between 40-60% ideally.
3. The melting temperatures of the primers should be between 42-65°C.
4. The final concentration to be used should be 0.1-0.5 µM of each primer.

Magnesium Concentration
1. The ideal magnesium concentration for Taq Polymerase is 1.5-2.0 mM.
2. The optimum concentration depends on the template, buffer, and dNTPs.
3. Higher than optimal concentration yields undesired products, and if concentration is too low, no amplification products are detected.

dNTPs
1. The typical concentration to be used is 200 µM.
2. Higher than optimal concentration of dNTPs yields higher yield but low fidelity.

Taq Polymerase
The typical concentration to be used for Taq Polymerase is 0.5 to 2 units per 50 µl of reaction.
PCR Reaction:
To set up a PCR reaction with Taq Polymerase:
1. Thaw all reaction components on ice.
2. Add Taq Polymerase at the end of the reaction.
3. Immediately transfer the tubes to a pre-heated thermal cycler once the reaction is set.
4. Start the reaction with desired cycling conditions with an annealing temperature set to 2°C difference of melting temperature between the forward and reverse primers.

Recomended PCR assay

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
10X Standard Taq Reaction Buffer	2.5 µl	5 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
Hot start Taq DNA Polymerase	0.65 µl	1.3 µl	1.5 units/50 µl PCR
Template DNA	variable	variable	<1,000 ng
Nuclease-Free Water	to 25 µl	to 50 µl	

Note: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Thermocycling Conditions for a Routine PCR:

STEP	TEMPERATURE	TIME
Initial Denaturation	95°C	10min
30 Cycles	95°C	15–30 seconds
	45–68°C	15–60 seconds
	72°C	1 minute/kb
Final Extension	72°C	10Min
Hold	4°C	∞

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