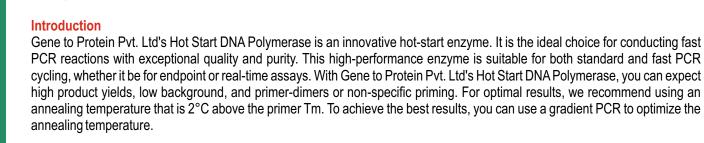


Hot Start Taq DNA Polymerase

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

Gene to Protein Pvt. Ltd. www.genetoprotein.com info@genetoprotein.com

(S)800 GENOME, 800 GENETIC



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	500U (2.5U/µL)- 200 µL	1000U (2.5U/µL)- 400 µL	100U (2.5U/µL)- 40 µL
Polymerase			
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, 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 10⁴ 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 $0.1-0.5 \,\mu$ M 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.
- 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.

	25 µl		FINAL
COMPONENT	REACTION	50 µl REACTION	CONCENTRATION
10X Standard Taq Reaction Buffer	2.5 µl	5 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 μΜ
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	

Recomended PCR assay

Thermocycling Conditions for a Routine PCR:

TEMPERATURE	TIME
95°C	10min
95°C	15-30 seconds
45–68°C	15–60 seconds
72°C	1 minute/kb
72°C	10Min
4°C	8
	95°C 95°C 45–68°C 72°C 72°C

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.

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