

Hot Start Taq DNA Polymerase



1-800-632-7799
info@neb.com
www.neb.com



M0495S



200 units **5,000 U/ml** **Lot: 0011212**
RECOMBINANT **Store at -20°C** **Exp: 12/14**

Description: Hot Start *Taq* DNA Polymerase is a mixture of *Taq* DNA Polymerase and an aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal cycling conditions allowing reactions to be set up at room temperature. This aptamer-based hot start does not require a separate high temperature incubation step to activate the enzyme. *Taq* DNA polymerase possesses a 5'→3' polymerase activity (1,2,3) and a 5' flap endonuclease activity (4,5).

It is supplied with 10X Standard *Taq* Reaction Buffer, which is detergent-free and designed to be compatible with existing assay systems.

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1

Application:

- High-specificity PCR
- Routine PCR
- Microarray Analysis
- Colony PCR

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, stabilizers and 50% glycerol

Reagents Supplied with Enzyme:
10X Standard *Taq* Reaction Buffer

Reaction Conditions: 1X Standard *Taq* Reaction Buffer, DNA template, primers, 200 μM dNTPs (not included) and 1.25 units of Hot Start *Taq* DNA Polymerase in a total reaction volume of 50 μl.

1X Standard *Taq* Reaction Buffer:

10 mM Tris-HCl
50 mM KCl
1.5 mM MgCl₂
pH 8.3 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 15 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.

Unit Assay Conditions: 1X ThermoPol™ Reaction Buffer, 200 μM dNTPs including [³H]-dTTP and 200 μg/ml activated Calf Thymus DNA.

Heat Inactivation: No

Quality Control Assays

High-Sensitivity PCR Assay: 30 cycles of PCR amplification of 20 pg Lambda DNA in a 50 μl reaction containing 100 ng human genomic DNA, 200 μM dNTPs, 0.2 μM primers, 1X ThermoPol Reaction Buffer and 1.25 units of Hot Start *Taq* DNA Polymerase result in a single 2 kb Lambda product. In non-Hot Start PCR control reactions, characteristic non-specific bands are produced from this set of primers and template.

3'→5' Exonuclease Activity: Incubation of a 20 μl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 10 nM fluorescent internally-labeled oligonucleotide for 30 minutes at either 37°C or 75°C yields no detectable 3'→5' degradation as determined by capillary electrophoresis.

Endonuclease Activity: Incubation of a 50 μl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 1 μg of supercoiled φX174 DNA for 4 hours at 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Inhibition Assay: Greater than 95% inhibition is observed after a 16 hour incubation at 25°C in a 50 μl primer extension assay containing 2.5 units of Hot Start *Taq* DNA Polymerase in 1X ThermoPol Reaction Buffer with 200 μM dNTPs including [³H]-dTTP and 15 nM primed single-stranded M13mp18.

PCR

The following guidelines are provided to ensure successful PCR using New England Biolabs' Hot Start *Taq* DNA Polymerase. These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure, low template concentrations, or amplicons greater than 5 kb may require further optimization.

Reaction setup:

Due to the hot start nature of the enzyme, reactions can be assembled on the bench at room temperature and transferred to a thermocycler. *No separate activation step is required to release the inhibitor from the enzyme.*

Add to a sterile thin-walled PCR tube:

COMPONENT	25 μl REACTION	50 μl REACTION	FINAL CONCENTRATION
10X Standard <i>Taq</i> 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 <i>Taq</i> DNA Polymerase	0.125 μl	0.25 μl	1.25 units/ 50 μl PCR
Template DNA	variable	variable	<1,000 ng
Nuclease-Free Water	to 25 μl	to 50 μl	

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary.

Transfer PCR tubes to a PCR machine and begin thermocycling:

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C	15–30 seconds
	45–68°C	15–60 seconds
	68°C	1 minute/kb
Final Extension	68°C	5 minutes
Hold	4–10°C	

General Guidelines:

1. Template:

Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 μl reaction are as follows:

DNA	AMOUNT
Genomic	1 ng–1 μg
Plasmid or Viral	1 pg–1 ng

2. Primers:

Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as PrimerSelect™ and Primer3 (http://frodo.wi.mit.edu/primer3_www.cgi) can be used to design or

analyze primers. The final concentration of each primer in a PCR may be 0.05–1 μM, typically 0.1–0.5 μM.

3. Mg⁺⁺ and additives:

Mg⁺⁺ concentration of 1.5–2.0 mM is optimal for most PCR products generated with Hot Start *Taq* DNA Polymerase. The final Mg⁺⁺ concentration in 1X Standard *Taq* Reaction Buffer is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg⁺⁺ can be further optimized in 0.5 or 1.0 mM increments using MgCl₂ (sold separately).

Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (6) or formamide (7).

4. Deoxynucleotides:

The final concentration of dNTPs is typically 200 μM of each deoxynucleotide.

5. Hot Start *Taq* DNA Polymerase Concentration:

We generally recommend using Hot Start *Taq* DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 μl reaction). However, the optimal concentration of Hot Start *Taq* DNA Polymerase may range from 5–50 units/ml (0.25–2.5 units/50 μl reaction) in specialized applications.

6. Denaturation:

No separate activation step is required to release the hot start inhibitor from the enzyme. An initial denaturation of 30 seconds at 95°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–4 minutes at 95°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minute incubation at 95°C is recommended to lyse cells.

During thermocycling a 15–30 second denaturation at 95°C is recommended.

7. Annealing:

The annealing step is typically 15–60 seconds. Annealing temperature is based on the T_m of the primer pair and is typically 45–68°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated T_m. We recommend using NEB's T_m Calculator, available at www.neb.com/TmCalculator to determine appropriate annealing temperatures for PCR.

(see other side)
CERTIFICATE OF ANALYSIS

When primers with annealing temperatures above 68°C are used, a 2-step PCR protocol is possible (see #10).

8. Extension:

The recommended extension temperature is 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°C is recommended.

9. Cycle number:

Generally, 25–35 cycles yield sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

10. 2-step PCR:

When primers with annealing temperatures above 68°C are used, a 2-step thermocycling protocol is possible.

Thermocycling Conditions for a Routine 2-Step PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C	15–30 seconds
	68°C	1 minute/kb
Final Extension	68°C	5 minutes
Hold	4–10°C	

11. PCR product:

The PCR products generated using Hot Start *Taq* DNA Polymerase contain dA overhangs at the 3' end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

References:

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- Lawyer, F.C. et al. (1993) *PCR Methods and Appl.*, 2, 275–287.
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- Lyamichev, V., Brow, M.A. and Dahlberg, J.E. (1993) *Science*, 260, 778–783.

- Sun, Y., Hegamyer, G. and Colburn, N. (1993) *Biotechniques*, 15, 372–374.
- Sarkar, G., Kapelner, S. and Sommer, S.S. (1990) *Nucleic Acids Res.*, 18, 7465.

Companion Products Sold Separately:

Hot Start *Taq* 2X Master Mix
 #M0496S 100 reactions
 #M0496L 500 reactions

Standard *Taq* Reaction Buffer Pack
 #B9014S 6.0 ml

Magnesium Chloride (MgCl₂) Solution
 #B9021S 6.0 ml

Deoxynucleotide Solution Set
 #N0446S 25 µmol each

Deoxynucleotide Solution Mix
 #N0447S 8 µmol each
 #N0447L 40 µmol each

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