One*Taq*® Hot Start DNA Polymerase

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M0481S 0051206614061

M0481S

200 units **5,000 U/ml** **Lot: 0051206**

RECOMBINANT **Store at –20°C** **Exp: 6/14**

Description: One*Taq* Hot Start DNA Polymerase is an optimized blend of *Taq* and Deep Vent$_R$™ DNA polymerases combined with an aptamer-based inhibitor. The 3´→ 5´ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robust amplification of *Taq* DNA Polymerase (1) and the hot start formulation combines convenience with decreased interference from primer dimers and secondary products. The One*Taq* Reaction Buffers and High GC Enhancer have been formulated for robust yields with minimal optimization, regardless of a template's GC content.

The inhibitor binds reversibly, blocking polymerase activity at temperatures below 45°C. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. One*Taq* Hot Start DNA Polymerase does not require a separate high temperature incubation step to activate the enzyme and can be used in typical *Taq*-based cycling protocols.

AMPLICON % GC CONTENT	RECOMMENDED DEFAULT BUFFER	OPTIMIZATION NOTES
≤ 50% GC	One*Taq* Standard Reaction Buffer	Adjust annealing temperature, primer/template concentration, etc. if needed.
50–65% GC	One*Taq* Standard Reaction Buffer	One*Taq* GC Reaction Buffer can be used to improve performance of difficult amplicons
> 65% GC	One*Taq* GC Reaction Buffer	10–20% One*Taq* High GC Enhancer may be added to reactions with One*Taq* GC Reaction Buffer to enhance performance of difficult amplicons

Notes: The OneTaq High GC Enhancer should not be used alone. It should be added only to the OneTaq GC Reaction Buffer and will typically enhance yields when other conditions have failed.

One*Taq* Hot Start DNA Polymerase is supplied with two 5X buffers: (Standard and GC), as well as a High GC Enhancer solution. For most routine and/or AT-rich amplicons (Lambda, etc.) or complex amplicons with up to ~65% GC content, One*Taq* Standard Reaction Buffer provides robust amplification. For GC-rich amplicons, the One*Taq* GC Reaction Buffer can improve both performance and yield. For particularly high GC or difficult amplicons (i.e. ≥ 75% GC), the One*Taq* High GC Enhancer can be added at a final concentration of 10–20% to reactions containing One*Taq* GC Reaction Buffer.

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1 and an *E. coli* strain that carries the Deep Vent$_R$ DNA Polymerase gene from *Pyrococcus* species GB-D.

Applications:

- High Sensitivity PCR
- High Throughput PCR
- Routine PCR
- GC-rich PCR
- AT-rich PCR
- Colony PCR
- Long PCR (up to ~6 kb genomic)

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween® 20, 0.5% IGEPAL® CA-630 and 50% glycerol

Reagents Supplied with Enzyme:
5X One*Taq* Standard Reaction Buffer
5X One*Taq* GC Reaction Buffer
One*Taq* High GC Enhancer

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

1X One*Taq* Standard Reaction Buffer:
20 mM Tris-HCl (pH 8.9 @ 25°C)
1.8 mM $MgCl_2$
22 mM NH_4Cl
22 mM KCl
0.06% IGEPAL CA-630
0.05% Tween 20

1X One*Taq* GC Reaction Buffer:
80 mM Tris-SO_4 (pH 9.2 @ 25°C)
2 mM $MgSO_4$
20 mM $(NH_4)_2SO_4$
5% Glycerol
5% DMSO
0.06% IGEPAL CA-630
0.05% Tween 20

One*Taq* High GC Enhancer:
10 mM Tris-HCl (pH 9.2 @ 25°C)
25% DMSO
25% Glycerol

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.

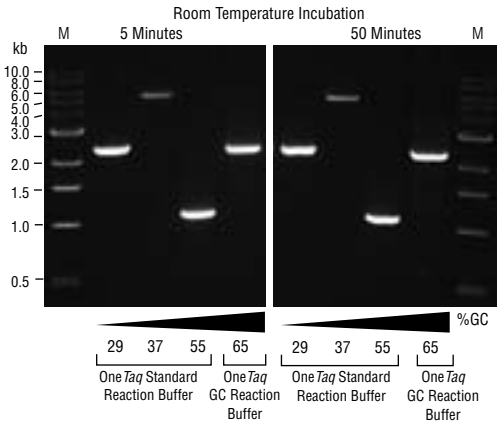
Unit Assay Conditions: 1X ThermoPol™ Reaction Buffer, 200 µM dNTPs including [^{3}H]-dTTP and 200 µg/ml activated Calf Thymus DNA.

Heat Inactivation: No

Quality Control Assays

5 kb Lambda PCR: 25 cycles of PCR amplification of 5 ng Lambda DNA with 0.625 units of One*Taq* Hot Start DNA Polymerase in a 25 µl reaction in the presence of 200 µM dNTPs, 0.2 µM primers and 1X One*Taq* Standard Reaction Buffer resulted in the expected 5 kb product.

High Sensitivity PCR Assay: 30 cycles of PCR amplification of 10 pg Lambda DNA with 0.625 units of One*Taq* Hot Start DNA polymerase in a 25 µl reaction containing 50 ng human genomic DNA, 200 µM dNTPs, 0.2 µM primers and 1X One*Taq* Standard Reaction Buffer resulted in a significant, enzyme dependent increase in the yield of the 2 kb Lambda product as compared to a standard PCR reaction. In non-hot start PCR control reactions, characteristic non-specific bands are produced from this set of primers and template.



Amplification of a selection of sequences with varying GC content from human and C. elegans genomic DNA using OneTaq Hot Start DNA Polymerase. The presence or absence of an extended room temperature incubation does not affect performance. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).

Buffer-dependent GC-rich (> 65% GC) PCR: 30 cycles of PCR amplification of 10 ng of human genomic DNA with 0.625 units of One*Taq* Hot Start DNA Polymerase in a 25 µl reaction in the presence of 200 µM dNTPs, 0.2 µM primers and 1X One*Taq* GC Reaction Buffer resulted in the buffer-dependent production of the 737 bp GC-rich product.

Enhancer-dependent High GC (> 70% GC) PCR: 30 cycles of PCR amplification of 10 ng of human genomic DNA with 0.625 units of One*Taq* Hot Start DNA Polymerase in a 25 µl reaction in the presence of 200 µM dNTPs, 0.2 µM primers, 20% One*Taq* High GC Enhancer and 1X One*Taq* GC Reaction Buffer resulted in the enhancer-dependent production of the 627 bp high GC product.

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 One*Taq* Hot Start DNA Polymerase in 1X ThermoPol Reaction Buffer with 200 µM dNTPs including [^{3}H]-dTTP and 15 nM primed single-stranded M13mp18.

Note: Product specifications for individual components in the One*Taq* Hot Start DNA Polymerase mix are available separately.

PCR

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (2). *Taq* DNA Polymerase is an enzyme widely used in PCR (3). One*Taq* Hot Start DNA Polymerase allows for greater amplification sensitivity across a wide variety of amplicons and increased ease of reaction setup. The following guidelines are provided to ensure successful PCR using New England Biolabs' One*Taq* Hot Start DNA Polymerase. These guidelines cover most routine PCR reactions. Specialized applications may require further optimization.

Reaction Setup:
Due to the presence of the inhibitor, 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.*

(see other side)

Add to a sterile thin-walled PCR tube:

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X One*Taq* Standard Reaction Buffer*	5 µl	10 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM
One*Taq* Hot Start 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	

*One*Taq* GC Reaction Buffer and High GC Enhancer can be used for difficult amplicons (see Description and General Guideline #3).

**For amplicons between 3–6 kb, use 2.5–5 units/50 µl rxn

Notes: 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.

Transfer PCR tubes to a PCR machine and begin thermocycling:

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	94°C	30 seconds
30 Cycles	94°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 reactions. 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™ (DNAStar Inc., Madison, WI) and Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. The final concentration of each primer in a PCR reaction may be 0.05–1 µM, typically 0.2 µM.

3. Mg^{++} and Additives:
   Mg^{++} concentration of 1.5–2.0 mM is optimal for most PCR products generated with One*Taq* Hot Start DNA Polymerase. The final Mg^{++} concentration in 1X One*Taq* Standard Reaction Buffer is 1.8 mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.2 mM increments using $MgCl_2$ (sold separately). One*Taq* (Mg-Free) Standard Reaction Buffer and supplemental $MgCl_2$ are also available separately for complete control of Mg^{++} concentration in the reaction.

   Amplification of some difficult targets, like GC-rich sequences, may be improved by the use of One*Taq* GC Reaction Buffer. The final Mg^{++} concentration in 1X One*Taq* GC Reaction Buffer is 2.0 mM. To optimize the Mg^{++} concentration of the One*Taq* GC Reaction Buffer, $MgSO_4$ should be used (sold separately). One*Taq* (Mg-Free) GC Reaction Buffer and supplemental $MgSO_4$ are also available separately for complete control of Mg^{++} concentration in the reaction.

   For extremely difficult amplicons, 10–20% One*Taq* High GC Enhancer can be added to reactions with One*Taq* GC Reaction Buffer. The enhancer should not be used alone and typically enhances yields when other conditions have failed.

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

5. One*Taq* Hot Start DNA Polymerase Concentration:
   We generally recommend using One*Taq* Hot Start DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 µl reaction) for amplicons up to 3 kb. The optimal concentration of One*Taq* Hot Start DNA Polymerase may range from 5–100 units/ml (0.25–5 units/50 µl reaction) for specialized applications. For 3–6 kb amplicons, 2.5–5 units/50 µl reaction is recommended. Note that in some cases, increasing the amount of enzyme in the reaction can be inhibitory.

6. Denaturation:
   No separate activation step is required to release the hot start inhibitor from the enzyme. An initial denaturation of 30 seconds at 94°C is sufficient to amplify most targets from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–4 minutes at 94°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 2–5 minute incubation at 94°C is recommended to lyse cells. During thermocycling a 10–30 second denaturation at 94°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.

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 yields sufficient product. Up to 45 cycles may be required to detect low copy number targets.

10. 2-step PCR:
    When primers with annealing temperatures of 68°C or above are used, a 2-step thermocycling protocol (combining annealing and extension into one step) is possible.

11. PCR Product:
    A significant portion of the PCR products generated using One*Taq* Hot Start DNA Polymerase contain dA overhangs at the 3´end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

References:

1. Barnes, W.M. (1994) *Proc. Natl. Acad. Sci. USA,* 91, 2216–2220.
2. Saiki R.K. et al. (1985) *Science,* 230, 1350–1354.
3. Powell, L.M. et al. (1987) *Cell,* 50, 831–840.

Companion Products Sold Separately:

One*Taq* DNA Polymerase
#M0480S 200 units
#M0480L 1,000 units
#M0480X 5,000 units

Diluent F
#B8006S 4.0 ml

One*Taq* Standard Reaction Buffer Pack
#B9022S 8.0 ml

One*Taq* GC Reaction Buffer Pack (includes One*Taq* High GC Enhancer)
#B9023S 8.0 ml

One*Taq* (Mg-free) Standard Reaction Buffer Pack
#B9024S 8.0 ml

Magnesium Chloride ($MgCl_2$) Solution
#B9021S 6.0 ml

One*Taq* (Mg-free) GC Reaction Buffer Pack (includes One*Taq* High GC Enhancer)
#B9025S 8.0 ml

Magnesium Sulfate ($MgSO_4$) Solution
#B1003S 6.0 ml

Deoxynucleotide Solution Set
#N0446S 25 µmol each

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

Notice to Purchaser:
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