One*Taq*® DNA Polymerase

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M0480S 009150317031

M0480S

200 units **5,000 U/ml** **Lot: 0091503**
RECOMBINANT **Store at –20°C** **Exp: 3/17**

Description: One*Taq* DNA Polymerase is an optimized blend of *Taq* and Deep Vent$_R$™ DNA polymerases for use with routine and difficult PCR experiments. The 3´→ 5´ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robust amplification of *Taq* DNA Polymerase (1). 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.

One*Taq* 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, the One*Taq* High GC Enhancer can be added at a final concentration of 10–20% to reactions containing One*Taq* GC Reaction Buffer.

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

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

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
- Primer Extension
- 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* 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 15 nM primed M13 DNA

Heat Inactivation: No

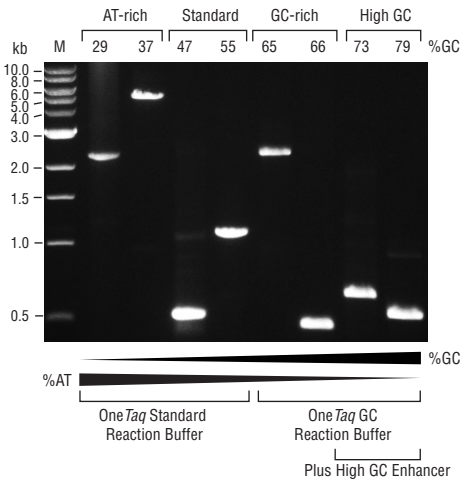
Quality Control Assays

5 kb Lambda PCR: 25 cycles of PCR amplification of 5 ng Lambda DNA with 0.625 units of One*Taq* 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.

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* 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* 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.

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



Amplification of a selection of sequences with varying GC content from human and C. elegans genomic DNA using OneTaq DNA Polymerase. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).

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* DNA Polymerase allows for greater amplification sensitivity across a wide variety of amplicons regardless of GC content. The following guidelines are provided to ensure successful PCR using New England Biolabs' One*Taq* DNA Polymerase. These guidelines cover most routine PCR reactions. Specialized applications may require further optimization.

Reaction Setup:

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (94°C).

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* 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	

(see other side)

CERTIFICATE OF ANALYSIS

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* 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 increases yields when other conditions have failed.

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

5. One*Taq* DNA Polymerase Concentration:
   We generally recommend using One*Taq* 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* DNA Polymerase may range from 5–100 units/ml (0.25–5 units/50 µl reaction). For specialized applications, including 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:
   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 15–30 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* 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* Hot Start DNA Polymerase
#M0481S 200 units
#M0481L 1,000 units
#M0481X 5,000 units

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

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices