One Taq DNA Polymerase

**Description:** One Taq DNA Polymerase is an optimized blend of Taq and Deep Vent™ DNA polymerases for use with routine and difficult PCR experiments. The 3′ → 5′ exo nuclease 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.

**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% IGEPA® 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 MgCl₂
- 22 mM NH₄Cl
- 22 mM KCl
- 0.06% IGEPA® CA-630
- 0.05% Tween 20

1X One Taq GC Reaction Buffer:
- 80 mM Tris-SO₄ (pH 9.2 @ 25°C)
- 2 mM MgSO₄
- 20 mM (NH₄)₂SO₄
- 5% Glycerol
- 5% DMSO
- 0.06% IGEPA® 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.

**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, DNA Polymerase gene from *Pyrococcus* species GB-D.

**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.

**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**

- 1X 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
- Template DNA variable variable <1,000 ng
- Nuclace-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).

**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:

<table>
<thead>
<tr>
<th>STEP</th>
<th>TEMP</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94°C</td>
<td>30 seconds</td>
</tr>
<tr>
<td>30 Cycles</td>
<td>94°C</td>
<td>15–30 seconds</td>
</tr>
<tr>
<td>45–68°C</td>
<td>15–60 seconds</td>
<td></td>
</tr>
<tr>
<td>Final Extension</td>
<td>68°C</td>
<td>1 minute/kb</td>
</tr>
<tr>
<td>Hold</td>
<td>4–10°C</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>

Amplification of a selection of sequences with varying GC content from human and C. elegans genomic DNA using One Taq DNA Polymerase. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #62232).
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:

<table>
<thead>
<tr>
<th>DNA</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic</td>
<td>1 ng–1 µg</td>
</tr>
<tr>
<td>Plasmid or Viral</td>
<td>1 pg–1 ng</td>
</tr>
</tbody>
</table>

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 OneTaq DNA Polymerase. The final Mg++ concentration in 1X OneTaq 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 MgCl₂ (sold separately). OneTaq (Mg-Free) Standard Reaction Buffer and supplemental MgCl₂ 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 OneTaq GC Reaction Buffer. The final Mg++ concentration in 1X OneTaq GC Reaction Buffer is 2.0 mM. To optimize the Mg++ concentration of the OneTaq GC Reaction Buffer, MgSO₄ should be used (sold separately). OneTaq (Mg-Free) GC Reaction Buffer and supplemental MgSO₄ are also available separately for complete control of Mg++ concentration in the reaction.

For extremely difficult amplicons, 10–20% OneTaq High GC Enhancer can be added to reactions with OneTaq 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. OneTaq DNA Polymerase Concentration:
We generally recommend using OneTaq 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 OneTaq 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 Tm 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 Tm. We recommend using NEB’s Tm 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 OneTaq DNA Polymerase contain dA overhangs at the 3’ end; therefore the PCR products can be ligated to dT/du-overhang vectors.

References:

Companion Products Sold Separately:

- OneTaq Hot Start DNA Polymerase
  - #M0481S 200 units
  - #M0481L 1,000 units
  - #M0481X 5,000 units

- OneTaq Standard Reaction Buffer Pack
  - #B9022S 8.0 ml

- OneTaq GC Reaction Buffer Pack
  - (includes OneTaq High GC Enhancer)
  - #B9023S 8.0 ml

- OneTaq (Mg-free) Standard Reaction Buffer Pack
  - #B9024S 8.0 ml

- Magnesium Chloride (MgCl₂) Solution
  - #B9021S 6.0 ml

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

- Magnesium Sulfate (MgSO₄) Solution
  - #B1003S 6.0 ml

- Deoxynucleotide Solution Set
  - #N0446S 25 µmol each

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