OneTag 2X Master Mix with GC Buffer

**Description:** OneTag 2X Master Mix with GC Buffer is an optimized blend of Taq and Deep Vent DNA Polymerases ideally suited to PCR applications from GC-rich templates, including pure DNA solutions, bacterial colonies, and cDNA products. The 3'→5' exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robust amplification of Taq DNA Polymerase. The convenient Master Mix formulation contains dNTPs, MgSO₄, and other buffer components and stabilizers listed below, requiring only the addition of primers and DNA template for robust amplification.

**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.

**Applications:**
- GC-rich PCR
- High Sensitivity PCR
- High Throughput PCR
- Colony PCR
- Long PCR (up to ~6 kb genomic)

**Reagents Supplied with Enzyme:**
- OneTag High GC Enhancer

**Reaction Conditions:** OneTag 2X Master Mix with GC Buffer, DNA template and primers in a total reaction volume of 50 µl.

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

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

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

4. **Heat Inactivation:** No

**Quality Control Assays**

- **Buffer-dependent GC-rich (> 65% GC) PCR:**
  - 30 cycles of PCR amplification of 10 ng of human genomic DNA with 1X OneTag Master Mix with GC Buffer in a 25 µl reaction in the presence of 0.2 µM primers 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 1X OneTag Master Mix with GC Buffer in a 25 µl reaction in the presence 0.2 µM primers and 20% OneTag High GC Enhancer resulted in the enhancer-dependent production of the 627 bp high GC product.

5. **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 Primer3 ([http://frodo.wi.mit.edu/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. **Mg²⁺ and Additives:**
      - Mg²⁺ concentration of 1.5–2.0 mM is optimal for most PCR products generated with OneTag DNA Polymerase. The final Mg²⁺ concentration in 1X OneTag Master Mix with GC Buffer is 2 mM. This supports satisfactory amplification of most amplicons. However, Mg²⁺ can be further optimized in 0.2 mM increments using MgSO₄ (sold separately).

   4. **Denaturation:**
      - An initial denaturation of 30 seconds at 94°C is sufficient to amplify most targets from pure DNA templates. For difficult templates, 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 (see other side)

**Amplification of a selection of sequences with varying GC content from human genomic DNA using OneTag 2X Master Mix with GC Buffer.** GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).
initial 2–5 minute denaturation at 94°C is recommended to lyse cells. During thermocycling a 15–30 second denaturation at 94°C is recommended.

5. 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 Tm Calculator, available at www.neb.com/TmCalculator to determine appropriate annealing temperatures for PCR.

6. 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.

7. Cycle Number:
Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low copy number targets.

8. 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.

9. PCR Product:
The majority 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.

Notes:
One Taq 2X Master Mix with GC Buffer is stable for fifteen freeze-thaw cycles when stored at –20°C.
One Taq 2X Master Mix with GC Buffer is also stable for one month at 4°C, so for frequent use, an aliquot may be kept at 4°C.

References:

Companion Products Sold Separately:
- Magnesium Sulfate (MgSO₄) Solution #B1003S 6.0 ml
- One Taq 2X Master Mix with Standard Buffer #M0482S 100 reactions #M0482L 500 reactions
- One Taq Quick-Load™ 2X Master Mix with Standard Buffer #M0486S 100 reactions #M0486L 500 reactions
- One Taq Quick-Load 2X Master Mix with GC Buffer #M0487S 100 reactions #M0487L 500 reactions
- One Taq DNA Polymerase #M0480S 200 units #M0480L 1,000 units #M0480X 5,000 units
- One Taq Hot Start DNA Polymerase #M0481S 200 units #M0481L 1,000 units #M0481X 5,000 units

ISO 9001
ISO 14001
ISO 13485
NEW ENGLAND BIOLABS®, ONETAQ®, QUICK-LOAD™ and THERMOPOL® are registered trademarks of New England Biolabs, Inc.
DEEP VENT™ is a trademark of New England Biolabs, Inc.
IGEPAL® is a registered trademark of Rhodia Operations.
TWEEN® is a registered trademark of Uniqema Americas LLC.