**Description:** One Taq Quick-Load 2X Master Mix with GC Buffer is an optimized, ready-to-use 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 5'-3' exonuclease activity of Deep Vent DNA Polymerase (1). The convenient quick-load master mix formulation contains dNTPs, MgSO4, buffer components and stabilizers as well as two commonly used tracking dyes for DNA gels. On a 1% agarose gel in 1X TBE, Xylene Cyanol FF migrates at ~4 kb and Tartrazine tracking dyes for DNA gels. On a 1% agarose gel in 1X TBE, Xylene Cyanol FF migrates at ~4 kb and on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature. Markers 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). The following guidelines are provided to ensure successful PCR using New England Biolabs’ One Taq Quick-Load 2X Master Mix with GC Buffer. These guidelines cover 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).

**Quality Control Assays**

Buffer-dependent GC-rich (> 65% GC) PCR:

30 cycles of PCR amplification of 10 ng of human genomic DNA with 1X One Taq Quick-Load 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 One Taq Quick-Load Master Mix with GC Buffer in a 25 µl reaction in the presence of 0.2 µM primers and 20% One Taq High GC Enhancer resulted in the enhancer-dependent production of the 627 bp High GC product.

**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 DNA</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) 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 Quick-Load Master Mix with GC Buffer is 2 mM. This supports satisfactory amplification of most amplicons. However, Mg++ can be further optimized in 0.2 mM increments using MgSO4 (sold separately).

   Amplification of extremely difficult targets may be improved by the addition of 10–20% One Taq High GC Enhancer (included).

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**Transfer PCR tubes to a PCR machine and begin thermocycling:**

<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>45–68°C</td>
<td>15–60 seconds</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>

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**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.

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**One Taq Quick-Load Master Mix with GC Buffer**

100 reactions (50 µl vol) Lot: 0131312

**Applications:**

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

**Reagents Supplied with Enzyme:**

One Taq High GC Enhancer

**Reaction Conditions:** 1X One Taq Quick-Load Master Mix with GC Buffer, DNA template and primers in a total reaction volume of 50 µl.
**Notes:**

One Tag Quick-Load 2X Master Mix with GC Buffer is stable for fifteen freeze-thaw cycles when stored at −20°C.

One Tag Quick-Load 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 Tag Quick-Load 2X Master Mix
  - with Standard Buffer
    - #M0486S 100 Reactions
    - #M0486L 500 Reactions

- One Tag Hot Start Quick-Load 2X Master Mix with Standard Buffer
  - #M0488S 100 Reactions
  - #M0488L 500 Reactions

- One Tag Hot Start Quick-Load 2X Master Mix with GC Buffer
  - #M0489S 100 Reactions
  - #M0489L 500 Reactions

- One Tag DNA Polymerase
  - #M0480S 200 units
  - #M0480L 1,000 units
  - #M0480X 5,000 units

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

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  - #M0488S 100 Reactions
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