**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 robustness of PCR amplification of GC-rich templates, including pure DNA solutions, bacterial colonies and cDNA products. 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.

**PCR**

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (2). The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (2). 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).

**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>94°C</td>
<td>15–30 seconds</td>
<td></td>
</tr>
<tr>
<td>30 Cycles</td>
<td>45–68°C</td>
<td>15–60 seconds</td>
</tr>
<tr>
<td>68°C</td>
<td>1 minute/kb</td>
<td></td>
</tr>
<tr>
<td>Final Extension</td>
<td>68°C</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Hold</td>
<td>4–10°C</td>
<td></td>
</tr>
</tbody>
</table>

**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:
   - Genomic: 1–10 ng
   - Plasmid or Viral: 1–1 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 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**²⁺ and Additives:
   - Mg²⁺ concentration of 1.5–2.0 mM is optimal for most PCR products generated with One Taq DNA Polymerase. The final Mg²⁺ concentration in 1X One Taq Quick-Load 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).
   - Amplification of extremely difficult targets may be improved by the addition of 10–20% One Taq High GC Enhancer (included).

**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.
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 initial 2–5 minute denaturation at 94°C is recommended. 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 $T_m$.

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 Quick-Load 2X Master Mix with GC Buffer is stable for fifteen freeze-thaw cycles when stored at −20°C.
One Taq 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 ($\text{MgSO}_4$) Solution
#B1003S 6.0 ml

One Taq™ Quick-Load® 2X Master Mix with Standard Buffer
#MO486S 100 Reactions
#MO486L 500 Reactions

One Taq™ Hot Start Quick-Load® 2X Master Mix with Standard Buffer
#MO488S 100 Reactions
#MO488L 500 Reactions

One Taq™ Hot Start Quick-Load® 2X Master Mix with GC Buffer
#MO489S 100 Reactions
#MO489L 500 Reactions

One Taq™ DNA Polymerase
#MO480S 200 units
#MO480L 1,000 units
#MO480X 5,000 units

One Taq™ Hot Start DNA Polymerase
#MO481S 200 units
#MO481L 1,000 units
#MO481X 5,000 units

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