1X One Taq Hot Start Master Mix with GC Buffer:
80 mM Tris-SO₄ (pH 9.2)
2 mM MgSO₄
20 mM (NH₄)₂SO₄
0.2 mM dNTPs
5% Glycerol
5% DMSO
0.06% IGEPA® CA-630
0.05% Tween-20
25 units/ml One Taq Hot Start DNA Polymerase

One Taq High GC Enhancer:
10 mM Tris-HCl
25% DMSO
25% Glycerol, pH 9.2 @ 25°C

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

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 One Taq Hot Start 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 Hot Start 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 GC-rich product.

Note: Product specifications for individual components in the One Taq Hot Start 2X Master Mix with GC Buffer are available separately.

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 Hot Start Master Mix with GC Buffer, DNA template and primers in a total reaction volume of 50 µl.

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 minutes</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>

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 Type</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) 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 One Taq DNA Polymerase. The final Mg²⁺ concentration in 1X One Taq Hot Start 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.

(see other side)
4. Denaturation:
No separate activation step is required to release the hot start inhibitor from the enzyme. 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 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.

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™ Hot Start 2X Master Mix with GC Buffer is stable for fifteen freeze-thaw cycles when stored at −20°C.

One Taq™ Hot Start 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™ Hot Start 2X Master Mix with Standard Buffer #M0484S 100 reactions #M0484L 500 reactions
One Taq™ Hot Start Quick-Load® 2X Master Mix with Standard Buffer #M0488S 100 reactions #M0488L 500 reactions
One Taq™ Hot Start Quick-Load® 2X Master Mix with GC Buffer #M0489S 100 reactions #M0489L 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

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