**Unit Assay 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**

5 kb Lambda PCR: 25 cycles of PCR amplification of 5 ng Lambda DNA with 1.25 units of Taq DNA Polymerase in the presence of 200 µM dNTPs and 0.2 µM primers in Standard Taq Reaction Buffer results in the expected 5 kb product.

3’→5’ Exonuclease Activity: Incubation of a 20 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of Taq DNA Polymerase with 10 nM fluorescent internally labeled oligonucleotide for 30 minutes at either 37°C or 75°C yields no detectable 3’→5’ degradation as determined by capillary electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of Taq DNA Polymerase with 1 µg of supercoiled phiX174 DNA for 4 hours at 75°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**PCRs**

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (6). Taq DNA Polymerase is an enzyme widely used in PCR (7). The following guidelines are provided to ensure successful PCR using New England Biolabs® Taq DNA Polymerase. These guidelines cover routine PCR reactions. Amplification of templates with high GC content, high secondary structure, low template concentrations, or amplicons greater than 5 kb 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 (95°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>95°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></td>
<td>68°C</td>
<td>1 minute/kb</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:

<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 0.1–0.5 µM.

3. **Mg²⁺ and additives:**

   Mg²⁺ concentration of 1.5–2.0 mM is optimal for most PCR products generated with Taq DNA Polymerase. The final Mg²⁺ concentration in 1X Standard Taq Reaction Buffer is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg²⁺ can be further optimized in 0.5 or 1.0 mM increments using MgCl₂.

   Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (8) or formamide (9).

4. **Deoxynucleotides:**

   The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

5. **Taq DNA Polymerase Concentration:**

   We generally recommend using Taq DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 µl reaction). However, the optimal concentration of Taq DNA Polymerase may range from 5–50 units/ml (0.25–2.5 units/50 µl reaction) in specialized applications.

6. **Denaturation:**

   An initial denaturation of 30 seconds at 95°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–4 minutes at 95°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minute denaturation at 95°C is recommended.

   During thermocycling a 15–30 second denaturation at 95°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.

   - When primers with annealing temperatures above 60°C are used, a 2-step PCR protocol is possible (see #10).

(see other side)
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 above 60°C are used, a 2-step thermocycling protocol is possible.

**Thermocycling Conditions for a Routine 2-Step PCR:**

<table>
<thead>
<tr>
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<tr>
<td>Hold</td>
<td></td>
<td>4–10°C</td>
</tr>
</tbody>
</table>

11. PCR product:
The PCR products generated using Taq 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:**
- Diluent F #B8006S 4.0 ml
- Standard Taq Reaction Buffer Pack #B9014S 6.0 ml
- Standard Taq (Mg-Free) Reaction Buffer Pack #B9015S 6.0 ml
- Magnesium Chloride (MgCl₂) Solution #B9021S 6.0 ml
- Taq PCR Kit #E5000S 200 Reactions
- Taq 2X Master Mix #M0270S 100 Reactions #M0270L 500 Reactions
- Quick-Load™ Taq 2X Master Mix #M0271S 100 Reactions #M0271L 500 Reactions
- Taq 5X Master Mix #M0285S 100 Reactions #M0285L 500 Reactions
- Deoxynucleotide Solution Set #N0446S 25 µmol each
- Deoxynucleotide Solution Mix #N0447S 8 µmol each #N0447L 40 µmol each

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