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ThermalAce™ DNA Polymerase

Cat. No.: Size:
E9200 200 Units
E1000 1000 Units

Conc: 2 U/μl Store at -20°C in a non-frost-free freezer

Description
ThermalAce™ DNA Polymerase is an extremely thermostable enzyme from a proprietary archaeobacterium that is specifically designed for high-yield PCR amplification of GC-rich templates (>65% GC content). The enzyme retains full activity after incubation at 95°C for 4 hours and has five-fold better processivity than Taq DNA polymerase. An optimized buffer reduces the need for additional optimization in many cases, making ThermalAce™ the enzyme of choice for a wide variety of applications. Sufficient reagents are provided for 100 or 500 amplification reactions of 50 μl each (at 2 units of ThermalAce™ DNA Polymerase per reaction).

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References
**Product Qualification**

The Certificate of Analysis (CoA) provides detailed quality control information for each product. The CoA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

**Recommendations and Guidelines**

**Template:** Use 10–100 ng genomic DNA, cDNA, or BAC DNA, or 1–10 ng plasmid DNA

**Primers:** Use 2100 ng each primer per 50-μl reaction. A T<sub>m</sub> of 65–70°C is optimal for most applications. Primer design is one of the most important factors in successful PCR. To design custom primers for your specific target sequence, we recommend the OligoPerfect™ Designer, available at www.invitrogen.com/oligos.

**Reaction:** Separate cycling programs are provided on page 3 for GC-rich templates (>65% GC content) and standard templates. Keep all components, reaction mixes, and samples on ice. After preparation of the samples, transfer them immediately to a preheated thermal cycler and start the amplification program.

Take appropriate precautions to avoid cross-contamination of DNA between reactions. Ideally, amplification reactions should be assembled in a DNA-free environment. Use of aerosol-resistant barrier tips is recommended. Avoid contamination with the primers or template DNA used in individual reactions. Analyze PCR products in an area separate from the reaction assembly area.

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**Protocol**

The following protocol is recommended as a starting point. Optimal reaction conditions (incubation times and temperatures; concentrations of enzyme, primers, and template) may vary, and may be adjusted as needed.

Recommended cycling temperatures are provided for both GC-rich templates (>65% GC content) and standard templates.

1. Add components in the following order to each reaction vessel on ice.
   - DNA template: x μl
   - Primers (100 ng each): 1 μl
   - 50X dNTP Mix (10 mM each dNTP): 1 μl
   - 10X ThermalAce™ Buffer: 5 μl
   - Sterile Water: to 49 μl
   - ThermalAce™ (2 U/μl)*: 1 μl
   - Final volume: 50 μl
   
   Note: Up to 3 U of enzyme (1.5 μl) may be added for difficult templates. A master mix can be prepared for multiple reactions to enable accurate pipetting.

2. Cap/seal the reaction vessels and flick with your finger for several seconds to mix. Place reaction(s) on ice until ready to cycle.

3. Program the thermal cycler as follows. Note that the annealing temperature may vary depending on the T<sub>m</sub> of your primers. The optimal annealing temperature is typically 5°C below the T<sub>m</sub> of the primers.

4. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use. Analyze 5–10 μl of sample by agarose gel electrophoresis.

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**Frequently Asked Questions**

**Denaturation:**
- Recommended: 98°C
- Temp range: 95°C – 100°C
- Extension: 1 min/kb

**Annealing:**
- Recommended: 65°C (5°C < T<sub>m</sub>)
- Temp range: 55°C – 65°C
- Extension: 1 min/kb

**Final Extension:**
- Recommended: 72°C
- Temp range: 70°C – 80°C
- Extension: 1 min/kb

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**Table:**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp (GC-rich template)</th>
<th>Temp (standard template)</th>
<th>Time Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>98°C</td>
<td>95°C</td>
<td>3 min</td>
</tr>
<tr>
<td>Denaturation</td>
<td>98°C</td>
<td>95°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>Annealing</td>
<td>65°C (5°C &lt; T&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>55°C (5°C &lt; T&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>30 sec</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>74°C</td>
<td>1 min/kb</td>
</tr>
<tr>
<td>Final Extension</td>
<td>72°C</td>
<td>74°C</td>
<td>10 min</td>
</tr>
</tbody>
</table>

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**Note:** Up to 3 U of enzyme (1.5 μl) may be added for difficult templates. A master mix can be prepared for multiple reactions to enable accurate pipetting.
Unit Definition
One unit of enzyme is the amount of enzyme required to incorporate 10 nmoles of dNTPs into acid insoluble material in 30 minutes at 74°C. This is the standard unit definition for all thermostable polymerases used for PCR.

Protocol
The following protocol is recommended as a starting point. Optimal reaction conditions (incubation times and temperatures; concentrations of enzyme, primers, and template) may vary, and may be adjusted as needed. Recommended cycling temperatures are provided for both GC-rich templates (>65% GC content) and standard templates.

1. Add components in the following order to each reaction vessel on ice.
   DNA template  x μl
   Primers (100 ng each) 1 μl
   50X dNTP Mix (10 mM each dNTP) 1 μl
   10X ThermalAce” Buffer 5 μl
   Sterile Water to 49 μl
   ThermalAce” (2 U/μl)* 1 μl
   Final volume 50 μl
   Note: Up to 3 U of enzyme (1.5 μl) may be added for difficult templates. A master mix can be prepared for multiple reactions to enable accurate pipetting.

2. Cap/seal the reaction vessels and flick with your finger for several seconds to mix. Place reaction(s) on ice until ready to cycle.

3. Program the thermal cycler as follows. Note that the annealing temperature may vary depending on the Tm of your primers. The optimal annealing temperature is typically 5°C below the Tm of the primers.

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<td>98°C</td>
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<td>3 min</td>
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<td>95°C</td>
<td>30 sec</td>
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<td>Annealing</td>
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<td>55°C (T&lt;sub&gt;a&lt;/sub&gt; &lt; Tm)</td>
<td>30 sec</td>
<td>25–30</td>
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<td>Extension</td>
<td>72°C</td>
<td>74°C</td>
<td>1 min/kb</td>
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4. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use. Analyze 5–10 μl of sample by agarose gel electrophoresis.
ThermalAce™ DNA Polymerase

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<tbody>
<tr>
<td>E0200</td>
<td>200 Units</td>
<td>2 U/μl</td>
<td>-20°C</td>
<td>500 μl</td>
</tr>
<tr>
<td>E1000</td>
<td>1000 Units</td>
<td></td>
<td></td>
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