**Product Information**

*Taq DNA Polymerase, from Thermus aquaticus recombinant, expressed in Escherichia coli* with 10x reaction buffer without MgCl$_2$

Catalog Number **D4545**
Storage Temperature −20 °C

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**TECHNICAL BULLETIN**

**Introduction**
*Taq* DNA Polymerase is a thermostable enzyme derived from the thermophilic bacterium *Thermus aquaticus*. The enzyme is in a recombinant form, expressed in *E. coli*. It is able to withstand repeated heating to 95 °C without significant loss of activity. The enzyme is ~94 kDa by SDS-PAGE with no detectable contaminating endonuclease or exonuclease activity. It has 5→3' DNA polymerase activity and 5'→3' exonuclease activity. Each lot of *Taq* DNA Polymerase is tested for PCR amplification and double-stranded sequencing. The enzyme is supplied at 5 units/µL and comes with an optimized 10× reaction buffer without magnesium chloride. A separate tube of magnesium chloride is included to allow its titration to optimal efficiency.

**Unit Definition:** One unit incorporates 10 nmol of total deoxyribonucleoside triphosphates into acid precipitable DNA in 30 minutes at 74 °C.

**Reagents Provided**
- *Taq* DNA Polymerase, Catalog No. D6677
  5 units/µL in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, stabilizers, 50% glycerol
- 10× PCR Buffer without MgCl$_2$, Catalog No. P2317, 100 mM Tris-HCl, pH 8.3, 500 mM KCl
- Magnesium chloride solution, 25 mM, Catalog No. M8787

**Reagents required but not provided**
- 10 mM dATP, Catalog No. D6920
- 10 mM dCTP, Catalog No. D7045
- 10 mM dGTP, Catalog No. D7170
- 10 mM TTP, Catalog No. T7791
- Deoxynucleotide Mix, Catalog No. D7295 containing 10 mM dATP, dCTP, dGTP, TTP
- Water, PCR Reagent, Catalog No. W1754
- Mineral Oil, Catalog No. M8662 (optional)
- Thermal cycler
- Primers
- DNA to be amplified

**Precautions and Disclaimer**
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Storage**
Store at −20 °C

**Amplification Procedure**
The optimal conditions for the concentration of *Taq* DNA polymerase, template DNA, primers, and MgCl$_2$ will depend on the system being utilized. It may be necessary to determine the optimal conditions for each individual component. This is especially true for the *Taq* DNA polymerase, cycling parameters, and the MgCl$_2$ concentration. It is recommended the enzyme and the MgCl$_2$ be titrated to determine the optimal efficiency.

1. Add the following reagents to a 200 or 500 µL microcentrifuge tube in the following order:

<table>
<thead>
<tr>
<th>Amount</th>
<th>Component</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>- µL</td>
<td>Water</td>
<td>-</td>
</tr>
<tr>
<td>5 µL</td>
<td>10x PCR Buffer</td>
<td>1x</td>
</tr>
<tr>
<td>- µL</td>
<td>25 mM MgCl$_2$</td>
<td>Typically 1.5-3.5 mM</td>
</tr>
<tr>
<td>1 µL*</td>
<td>10 mM dATP</td>
<td>200 µM</td>
</tr>
<tr>
<td>1 µL*</td>
<td>10 mM dCTP</td>
<td>200 µM</td>
</tr>
<tr>
<td>1 µL*</td>
<td>10 mM dGTP</td>
<td>200 µM</td>
</tr>
<tr>
<td>1 µL*</td>
<td>10 mM TTP</td>
<td>200 µM</td>
</tr>
<tr>
<td>- µL</td>
<td>Forward primer (typically 15-30 bases in length)</td>
<td>0.1-0.5 µM</td>
</tr>
<tr>
<td>- µL</td>
<td>Reverse primer (typically 15-30 bases in length)</td>
<td>0.1-0.5 µM</td>
</tr>
<tr>
<td>0.5 µL</td>
<td><em>Taq</em> DNA Polymerase</td>
<td>0.05 units/µL</td>
</tr>
<tr>
<td>- µL</td>
<td>Template DNA (typically 10 ng)</td>
<td>200 pg/µL</td>
</tr>
<tr>
<td>50 µL</td>
<td>Final volume</td>
<td></td>
</tr>
</tbody>
</table>
*Note: The individual nucleotides (4 µL total) may be substituted by 1 µL of Deoxynucleotide Mix, Catalog No. D7295.

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.
3. Add 100 µL of mineral oil to the top of each tube to prevent evaporation if using a thermal cycler without a heated lid.
4. The amplification parameters will vary depending on the primers and the thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

<table>
<thead>
<tr>
<th>Denature the template</th>
<th>94 °C</th>
<th>1 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anneal primers</td>
<td>55 °C</td>
<td>2 min</td>
</tr>
<tr>
<td>Extension</td>
<td>72 °C</td>
<td>3 min</td>
</tr>
<tr>
<td>25-30 cycles of amplification are recommended</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. The amplified DNA can be evaluated by agarose gel electrophoresis and subsequent ethidium bromide staining. Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase.

**References**


**Related Products**

**Reagents**

- Lambda DNA Hind III Digest, Catalog No. D9780
- Enhanced Avian HS RT-PCR kits, Catalog No HSRT100 (100 reactions).

**Equipment**

- PCR Multiwell Plate, 96-well, Catalog No. Z374903
- PCR Multiwell Plate, 384-well, Catalog No. Z374911
- PCR Microtubes, 0.2 ml, attached caps, Catalog No. Z374873
- PCR Microtubes, 0.2 ml strip tubes with strip caps, Catalog No. Z374962
- Sealing accessory for PCR vessels, Micro Mats, Catalog No. Z374938
- PCR Workstation, 120V, Catalog No. Z376213
- PCR Workstation, 240V, Catalog No. Z376221

**NOTICE TO PURCHASER: LIMITED LICENSE**

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