PRODUCT INFORMATION
T4 DNA Polymerase

Pub. No. MAN0012013
Rev. Date 13 June 2016 (B.00)

Lot: _  Expiry Date: _

<table>
<thead>
<tr>
<th>Components</th>
<th>#EP0061</th>
<th>#EP0062</th>
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<tbody>
<tr>
<td></td>
<td>100 U</td>
<td>500 U</td>
</tr>
<tr>
<td>Concentration</td>
<td>5 U/µL</td>
<td>5 U/µL</td>
</tr>
<tr>
<td>5X Reaction Buffer</td>
<td>0.35 mL</td>
<td>2 × 1 mL</td>
</tr>
</tbody>
</table>

Store at -20 °C

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Description
T4 DNA Polymerase, a template-depended DNA polymerase, catalyzes 5'→3' synthesis from primed single-stranded DNA. The enzyme has a 3'→5' exonuclease activity, but lacks 5'→3' exonuclease activity.

Applications
- Blunting of DNA ends: fill-in 5'-overhangs or/and removal of 3'-overhangs (1, 2), see protocol on back page.
- Blunting of PCR products with 3'-dA overhangs (6).
- Synthesis of labeled DNA probes by the replacement reaction (3).
- Oligonucleotide-directed site-specific mutagenesis (4).
- Ligation-independent cloning of PCR products (5).

Source
E.coli cells with a cloned gene 43 of bacteriophage T4.

Molecular Weight
104 kDa monomer.

Definition of Activity Unit
One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 37 °C.
**Storage Buffer**  
The enzyme is supplied in: 20 mM potassium phosphate (pH 7.5), 200 mM KCl, 2 mM DTT, and 50% (v/v) glycerol.

**5X Reaction Buffer**  
335 mM Tris-HCl (pH 8.8 at 25 °C), 33 mM MgCl₂, 5 mM DTT, 84 mM (NH₄)₂SO₄.

**Inhibition and Inactivation**  
- Inhibitors: metal chelators, nucleotide analogs  
  2(p-n-butylanilino)-dATP, N²-(p-n-butylyphenyl)-dGTP,  
  SH-blocking compounds (7).  
- Inactivated by heating at 75 °C for 10 min.

**Note**  
- The 3'→5' exonuclease activity of T4 DNA Polymerase is stronger on single-stranded DNA than on double-stranded DNA and greater (more than 200 times) than that of DNA Polymerase I, *E.coli* (1).
- Activity in Thermo Scientific buffers, %  
  (in comparison to activity in assay buffer)

<table>
<thead>
<tr>
<th>Buffers</th>
<th>Activity, %</th>
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</table>
| for restriction enzymes:  
  Thermo Scientific™ FastDigest™, FastDigest Green, O, R, 1X Thermo Scientific™ Tango™, 2X Tango, BamHI, EcoRI, Ecl136II, KpnI, PaeI, Sacl, B, G | 100 |
| | 75-100 |
| for PCR buffers:  
  *Taq* buffer with KCl and *Pfu* buffer | 50 |
| *Taq* buffer with (NH₄)₂SO₄ | 100 |
| RT buffers | 100 |

**CERTIFICATE OF ANALYSIS**

**Endodeoxyribonuclease Assay**  
No detectable degradation was observed after incubation of supercoiled plasmid DNA with T4 DNA Polymerase.

Quality authorized by:  
Jurgita Zilinskiene

(continued on back page)
Protocol for blunting of 5’- or 3'-overhangs

1. Prepare the following reaction mixture:

<table>
<thead>
<tr>
<th></th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>5X reaction buffer</td>
<td>4 µL</td>
</tr>
<tr>
<td>Linear DNA or PCR product</td>
<td>1 µg</td>
</tr>
<tr>
<td>dNTP Mix, 2 mM each (#R0241)</td>
<td>1 µL (0.1 mM final concentration)</td>
</tr>
<tr>
<td>T4 DNA Polymerase</td>
<td>0.2 µL (1 U)</td>
</tr>
<tr>
<td>Water, nuclease-free (#R0581)</td>
<td>to 20 µL</td>
</tr>
</tbody>
</table>

2. Mix thoroughly, spin briefly and incubate at 11 °C for 20 min or at room temperature for 5 min.
3. Stop the reaction by heating at 75 °C for 10 min.

References

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