T4 DNA Polymerase
(3,000 U/ml)

Catalog: RK20523
Size: 150 U / 750 U
Concentration: 3,000 U/ml
Components:

T4 DNA Polymerase (3,000 U/ml)	RM20513
10X ABuffer B	RM20126

Product Description

T4 DNA Polymerase catalyzes the synthesis of DNA in the 5´→ 3´ direction and requires the presence of template and primer. This enzyme has a 3´→ 5´ exonuclease activity which is much more active than that found in DNA Polymerase I (*E.coli*). Unlike *E.coli* DNA Polymerase I, T4 DNA Polymerase does not have a 5´→ 3´ exonuclease function.
It is applicable to 3´ overhang removal to form blunt ends, 5´ overhang fill-in to form blunt ends, single strand deletion subcloning, second strand synthesis in site-directed mutagenesis, and probe labeling using replacement synthesis.

Product Source: Purified from a strain of *E.coli* that carries the T4 DNA Polymerase gene.
Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C.

Storage Conditions: 100 mM KPO_4, 1 mM DTT, 50% Glycerol, pH 6.5 @ 25°C
Storage Temperature: -20°C

Reaction Conditions: 1X ABuffer B, Incubate at 12°C.
1X ABuffer B: 10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH7.9 @ 25°C
Activity in ABclonal Buffer

ABufferA	ABufferB	ABufferC	ABufferS
60%	100%	100%	100%

Heat Inactivation: 75°C for 20 min
Molecular Weight: Theoretical 104000 daltons
5' - 3' Exonuclease: No
3' - 5' Exonuclease: Yes
Strand Displacement: No
Error Rate: ~ 1 x 10^{-6} bases

Instructions

Protocol for blunting ends by 3' overhang removal and 3' recessed (5' overhang) end fill-in using T4 DNA Polymerase:

1. DNA should be dissolved in 1X reaction buffer* supplemented with 100 µM dNTPs.
2. Add 1 unit T4 DNA Polymerase per microgram DNA.
3. Incubate 15 minutes at 12°C.
4. Stop reaction by adding EDTA to a final concentration of 10 mM and heating to 75°C for 20 minutes.

CAUTION: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3´ → 5´ exonuclease activity of the enzyme.

**T4 DNA Polymerase can be used in CutA, CutB and CutS as well as ABuffer A/B/S and T4 DNA Ligase Reaction Buffer. Optimal activity is observed in ABufferB. BSA supplementation is recommended when using a buffer that does not already contain BSA.*

Notes:

1. For fill-in reactions only: T4 DNA Polymerase can be used in CutA, CutB and CutS as well as ABuffer A/B/S and T4 DNA Ligase Reaction Buffer.
2. For blunting reactions requiring removal of overhangs: T4 DNA Polymerase can be used in CutA, CutB and CutS as well as ABuffer A/B/S and T4 DNA Ligase Reaction Buffer. CutC and ABuffer C are not recommended when overhang removal is required.
3. Optimal activity is observed in ABuffer B.
4. Supplement with dNTPs*.
5. BSA supplementation is recommended when using a buffer that does not already contain BSA.
6. Incubate at temperature suggested for specific protocol.

** Refer to specific protocol to determine recommended dNTP concentrations.*

QC Process:

- Purity (SDS-PAGE) is above 95%.

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- No nuclease or RNase contamination.
- No residual host genomic DNA detected by PCR