

Product components

Components	Component number	Size-1	Size-2
		200 U	1000 U
T4 DNA Polymerase (5,000 U/mL)	RM21302	40µL	200µL
10X Buffer CutB	RM20105	1.25 mL	1.25 mL X 2

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 Applications

3' overhang removal to form blunt ends and 5' overhang fill-in to form blunt ends
 Probe labeling using replacement synthesis
 Second strand synthesis in site-directed mutagenesis
 Single strand deletion subcloning

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.

Reaction Conditions

10X Buffer CutB, Incubate at 12°C.

1X Buffer CutB

50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 0.1 mg/ml rHSA, pH 7.9 @ 25°C

Storage Temperature: -20°C

Storage Conditions

100 mM KPO₄, 1 mM DTT, 50% Glycerol, pH 6.5 @ 25°C

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

CAUTION:

1. 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.
2. 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 1X Buffer CutB. BSA supplementation is recommended when using a buffer that does not already contain BSA.
3. Refer to specific protocol to determine recommended dNTP concentrations.