T4 PDG (T4 Endonuclease V)

1-800-632-7799
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M0308S 005160618061

M0308S

RR BSA 37°

2,000 units **10,000 U/ml** **Lot: 0051606**

RECOMBINANT **Store at –20°C** **Exp: 6/18**

Description: T4 PDG (pyrimidine dimer glycosylase) has both DNA glycosylase and APlyase activity. The 16 kd protein recognizes cis-syn-cyclobutane pyrimidine dimers caused by UV irradiation. The enzyme cleaves the glycosyl bond of the 5´ end of the pyrimidine dimer and the endonucleolytic activity cleaves the phosphodiester bond at the AP site.

Source: Purified from an *E. coli* strain carrying a plasmid encoding T4 *denV* gene

Applications:

- DNA damage studies
- Single cell gel eletrophoresis (comet assay)

Supplied in: 100 mM NaCl, 10 mM Tris-HCl (pH 7.5 @25°C), 0.1 mM EDTA, 0.1 mM dithiothreitol and 50% glycerol.

Reagents Supplied with Enzyme:
10X T4 PDG Reaction Buffer,
100X BSA

Reaction Conditions:
1X T4 PDG Reaction Buffer, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X T4 PDG Reaction Buffer:
25 mM Na_2PO_4 (pH 7.2)
100 mM NaCl
1 mM EDTA
1 mM DTT

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the conversion of 0.5 µg of UV irradiated supercoiled pUC19 DNA to > 95% nicked plasmid in a total reaction volume of 20 µl in 30 minutes at 37°C. Nicking is assessed by agarose gel electrophoresis. Irradiated plasmid contains an average of 3–5 pyrimidine dimers.

Unit Assay Conditions: 1X T4 PDG Reaction Buffer containing 0.5 µg of UV irradiated supercoiled pUC19 DNA, supplemented with 100 µg/ml BSA in a 20 µl reation.

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (Hind III digest) and 100 units of T4 PDG (T4 Endonuclease V) for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 100 units of T4 PDG (T4 Endonuclease V) with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (10^5 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Note: For best results incubation time should be 30 minutes or less.

Addition of 1 µl of phenol to the sample before loading will improve electrophoresis by stripping the protein from the DNA.

Warm buffer to room temperature as it precipitates at 4°C.

References:

1. Higgins, K.L. and Lloyd, R.S. (1987) Mutation Research, 183, p. 117–121.

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS