

T4 PDG (T4 Endonuclease V)



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M0308S 004120714071

M0308S

RR BSA 37°

2,000 units 10,000 U/ml Lot: 0041207

RECOMBINANT Store at -20°C Exp: 7/14

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 electrophoresis (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 Na₂PO₄ (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 reaction.

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 [³H] *E. coli* DNA (10⁵ 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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