

TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

E. coli Photolyase

Catalog #:	4145-100-EB	Size:	
Contents:	4145-100-01 <i>E. coli</i> Photolyase	2,000 Units	
	4145-100-02 100 mM DTT	500 µl	
	3900-500-14 10X REC™ Buffer 14	1 ml	

Description: *E. coli* photolyase repairs *cis-syn* cyclobutane pyrimidine dimers (CPD) in UV irradiated DNA: light energy drives electron transport from a catalytic chromophore, reduced flavin adenine dinucleotide (FADH⁻), to the pyrimidine dimer, leading to photolysis of CPD. Photolyase is a monomeric protein of 471 amino acids (MW 53,994), and includes two noncovalently attached co-factors, the blue light harvest cofactor methenyltetrahydrofolate (MTHF) and the catalytic cofactor FADH⁻. Photolyase selectively binds to CPD in a light independent step. The MTHF cofactor absorbs at near UV-visible light (300-500 nm), and excites FADH⁻ by energy transfer. The excited FADH⁻, transfers an electron to split the CPD to regenerate the pyrimidines. The electron is transferred back to the photolyase, which causes the enzyme to dissociate from the DNA.

Source: Recombinant *E. coli* containing a recombinant plasmid harboring the *E. coli* photolyase *phr* gene.

Specificity: *Cis-syn* cyclobutane pyrimidine dimers.

Unit Definition: One unit will repair 50% of 1 µg supercoiled plasmid irradiated with 100 J/m² UV-light in one hour at 37 °C during photoreactivation by 365 nm blue light at the rate of 2 mW/cm. The repair is measured as a loss in conversion of the supercoiled plasmid to relaxed, open circle form following treatment with the CPD-specific endonuclease T4-PDG (T4-Endonuclease V).

Assay Conditions & Analysis:

UV-irradiation:

250 ng/µl of a 3,218 bp supercoiled plasmid, containing 50 potential CPD sites, was UV-irradiated with 100 J/m² of light at 254 nm, on ice.

Photolyase Treatment:

1.0 µg of plasmid UV-irradiated with 0 J/m² and 100 J/m² were incubated with and without photolyase in 1X REC Buffer 14 and 10 mM DTT in the dark for 5 minutes at room temperature in a 20 µl reaction volume. Reactions were then incubated under a near UV-visible light (75W GE black-light) for one hour at room temperature.

Detection of Repair:

250 ng of each sample of supercoiled plasmid was incubated with and without 10 units of T4 Endonuclease V (cat# 4055-100-01), 1X REC Buffer 11 (12.5 mM Na₂HPO₄, 12.5 mM NaH₂PO₄, 1 mM EDTA, 0.1 mM DTT, and 100 mM NaCl) at 37 °C for one hour. Reactions were resolved by electrophoresis on a 1% Trevigen 5000 gel (Cat# 9806-050-P) in 1X TAE buffer and 20 ng/ml ethidium bromide, and visualized under UV-light.

Storage Buffer: 20 mM Tris-Cl (pH 7.8), 50 mM NaCl, 1 mM EDTA, 10 mM DTT, and 50% (v/v) glycerol.

Storage Conditions: Store in working aliquots at -80 °C in a manual defrost freezer. May be diluted in 20 mM Tris-Cl (pH 7.8), 50 mM NaCl, 1 mM EDTA, 10 mM DTT, 0.1 mg/ml BSA, and 50% glycerol and stored at -20 °C for up to 1 week. Otherwise, dilute enzyme in 1X REC Buffer 14 and use immediately.

References:

- Cheung, M. S., I. Daizadeh, A. A. Stuchebrukhov, and P. F. Heelis. 1999. Pathways of electron transfer in *Escherichia coli* DNA photolyase: Trp306 to FADH. *Biophys. J.* **76**:1241-1249.
- Sancar, A., F. W. Smith, and G. B. Sancar. 1984. Purification of *Escherichia coli* DNA photolyase. *J. Biol. Chem.* **259**:6028-6032.
- Sancar, A. Structure and function of DNA Photolyase. 1994. *Biochemistry* **33**:2-9.
- Thoma, F. 1999. Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair. *EMBO J.* **18**:6585-6598.
- Jiang, Y., K. Changhong, P. A. Mieczkowski, and P. E. Marszalek. 2007. Detecting ultraviolet damage in single DNA molecules by atomic force microscopy. *Biophys. J.* **93**:1758-1767.

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Storage: -80 °C

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