

E.coli DNA Photolyase (Cyclobutane Pyrimidine Dimer Photolyase) 01-013 20 µg

Shipping and Storage: Ship at 4°C or -20°C and store at -20°C. Do not repeat freeze-thaw cycle

Product: Purified recombinant full-size E. coli Photolyase (Deoxyribodipyrimidine photo-lyase) described in Sancar А, Smith FW, Sancar GB. as Purification of Escherichia coli DNA photolyase J Biol **Chem.** 1984 May 10;259(9):6028-32. PMID: 6325459

Applications:

- 1. SDS-PAGE
- 2. Western blot
- 3. Immunoprecipitation
- 4. ELISA
- 5. Functional Studies

Form: 0.4 mg/ml in 50 mM KCl, 25 mM Tris-HCI, pH 7.4, 0.5 mM EDTA, 5 mM β -mercaptoethanol, 50% Glycerol.

Background: Involved in repair of UV radiation-induced DNA damage. Catalyzes the light-dependent monomerization (300-600 nm) of cyclobutyl pyrimidine dimers (in cis-syn configuration), which are formed between adjacent bases on the same DNA strand upon exposure to ultraviolet radiation. Upon absorption of visible light electrons are transferred from Trp-307 through Trp-360 to Trp 383, and from there to FADH, giving rise to the fully reduced catalytic FADH⁻. Length:472 aa. Mass (Da):53,667

Data Link: UniProtKB P00914 (PHR_ECOLI)



Fig 1. SDS-PAGE analysis of E. coli Photolyase

One μ g of Photolyase was applied and analyzed on 12% SDS-PAEG.





Fig.2 Western blot of E. coli photolyase

Samples

1. Purified Photolyase 8.4 ng

2. Purified Photolyase 42 ng

3. E. coli crude lysate

SDS-PAGE 12.5% gel

Blotting: 15 v, overnight, Bloking by 5% skim milk

Anti-Photolyase antibody used at 1/1,000 dilution.

 $2^{\rm nd}$ antibody: Goat anti-rabbit IgG (H & L) (ab97051) used at 1/10,000.

Note that there are only 10-20 molecules of photolyase per E.coli cell.



Fig 3 Immunoprecipitationm of E. coli Photolyase

Immunoprecipitation was performed by using Dynabeads Magnetic system (Thermo Fisher).

- 1. E. coli AB1157 cell lysate (300 μ g), antibody 2 μ g
- 2. E. coli AB1157 cell lysate (300 μ g), antibody 10 μ g
- 3. Positve control: E. coli Photolyase purified, $4.2\,\mu$ g, antibody $10\,\mu$ g

4. Negative control: No sample, antibody $10\,\mu\,{\rm g}$

Western blot: Anti-Photolyase antibody used at 1/1,000 dilution and as the second antibody, HRP-conjugated goat anti-rabbit IgG VeriBlot for IP (ab131366) was used at 1/1,000 dilution.

Related Product: 61-013 Anti- Cyclobutane Pyrimidine Dimer Photolyase (E. coli) antibody