Uracil-DNA Glycosylase (UDG)

1-800-632-7799
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M0280S 012130915091

M0280S

RR 37° No

1,000 units **5,000 U/ml** **Lot: 0121309**

RECOMBINANT **Store at –20°C** **Exp: 9/15**

Description: *E. coli* Uracil-DNA Glycosylase (UDG) catalyzes the release of free uracil from uracil-containing DNA. UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA, but not from oligomers (6 or fewer bases).

Source: An *E. coli* strain that carries the cloned UDG gene from *E. coli*.

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1 mg/ml BSA and 50% glycerol.

More Units

Application: Treatment of 0.1 µg of uracil-containing DNA with 1 unit of UDG for 10 minutes at 37°C renders the DNA incapable of being copied by DNA polymerase. The enzyme can be 95% heat killed by incubation at 95°C for 10 minutes. Since UDG remains partially active following heat treatment at 95°C, it is recommended that uracil glycosylase inhibitor be added to prevent degradation of product DNA. Alternatively, reaction products can be immediately extracted with phenol/chloroform.

Reagents Supplied with Enzyme:
10X UDG Reaction Buffer.

Reaction Conditions: 1X UDG Reaction Buffer. Incubate at 37°C.

1X UDG Reaction Buffer:
20 mM Tris-HCl
1 mM EDTA
1 mM dithiothreitol
(pH 8.0 @ 25°C).

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10^4–10^5 cpm/µg) in 30 minutes at 37°C.

Unit Assay Conditions: 1X UDG Reaction Buffer, 1 unit of uracil DNA Glycosylase, 0.2 µg ^{3}H-uracil DNA (10^4–10^5 cpm/µg) for 30 minutes at 37°C in a total reaction volume of 50 µl.

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ phage DNA in NEBuffer 1 and 50 units of UDG showed no degradation following overnight incubation at 37°C.

Exonuclease Activity: Incubation of 50 units for 4 hours at 37°C in 50 µl of NEBuffer 1 with 1 µg ^{3}H DNA (10^5 cpm/µg) released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 50 units of UDG with 1 µg of φX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 5% conversion to RF II.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection. BSA is added to the enzyme for stability.

Heat Inactivation: No

Notes On Use: UDG is active over a broad pH range with an optimum at pH 8.0, does not require divalent cation, and is inhibited by high ionic strength (> 200 mM).

References:

1. Lindahl, T. et al. (1977) *J. Biol. Chem.* 252, 3286–3294.
2. Wang, Z. et al. (1991) *Gene* 99, 31–37.
3. Devchand, P.R. et al. (1993) *Nucl. Acids Res.* 21, 3437–3443.

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