Uracil-DNA Glycosylase (UDG)

1-800-632-7799
info@neb.com
www.neb.com

M0280S 012160418041

M0280S

1,000 units **5,000 U/ml** **Lot: 0121604**

RECOMBINANT **Store at –20°C** **Exp: 4/18**

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.

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.

CERTIFICATE OF ANALYSIS

Uracil-DNA Glycosylase (UDG)

1-800-632-7799
info@neb.com
www.neb.com

M0280S 012160418041

M0280S

1,000 units 5,000 U/ml Lot: 0121604

RECOMBINANT Store at –20°C Exp: 4/18

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.

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.

CERTIFICATE OF ANALYSIS

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.