

Antarctic Thermolabile UDG



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M0372S 001131114111

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100 units **1,000 U/ml** **Lot: 00113111**

RECOMBINANT **Store at -20°C** **Exp: 11/14**

Description: Antarctic Thermolabile UDG (Uracil-DNA Glycosylase) catalyzes the release of free uracil from uracil-containing single-stranded or double-stranded DNA. The resulting abasic sites are susceptible to hydrolytic cleavage at elevated temperature and high pH. This enzyme is sensitive to heat and can be rapidly and completely inactivated at temperatures above 50°C.

Source: An *E. coli* strain that carries the cloned UDG gene from a psychrophilic marine bacterium

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Supplied in: 20 mM Tris-HCl (pH 7.5 @ 25°C), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

Applications:

- Prevention of Carry-over Contamination in PCR (1)
- Remove Uracil-base from DNA

Reagents Supplied with Enzyme:

10X Standard *Taq* Reaction Buffer.

Reaction Conditions:

1X Standard *Taq* Reaction Buffer. Incubate at 37°C.

1X Standard *Taq* Reaction Buffer:

10 mM Tris-HCl
1.5 mM MgCl₂
50 mM KCl
pH 8.3 @ 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 [³H]-uracil in a 50 µl Standard *Taq* Reaction Buffer containing 0.2 µg DNA (10⁴-10⁵ cpm/µg) in 30 minutes at 37°C.

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Unit Assay Conditions: 1X *Taq* Reaction Buffer, 1 unit of uracil DNA Glycosylase, 0.2 µg ³H-uracil DNA (10⁴-10⁵ cpm/µg) for 30 minutes at 37°C in a total reaction volume of 50 µl.

Quality Assurance: Antarctic Thermolabile UDG passes stringent quality control assays to ensure the highest level of functionality and purity. Please consult the product page at www.neb.com for more information.

Usage Notes: One unit of enzyme is capable of converting 2.3 nmol of 5' FAM-labeled 26-mer ssDNA with a single uracil to 10-mer ssDNA in 30 minutes at 37°C following NaOH and heat treatment. Activity is performed in a 50 µl standard *Taq* reaction buffer containing 2 pmol of 5' FAM-labeled 26-mer ssDNA with a single uracil and variable amount of enzyme in 30 minutes at 37°C.

The NEB unit is 2-5 fold more active per unit than other suppliers. This Antarctic Thermolabile UDG is active in most PCR reaction buffers but is inhibited with increasing ionic strength (> 100 mM).

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Quality Control Assays

16-Hour Incubation: A 50 µl reaction in Standard *Taq* Reaction Buffer containing 1 µg of HindIII-cut λ phage DNA and 50 units of Antarctic Thermolabile UDG incubated 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 in Standard *Taq* Reaction Buffer containing 50 units of Antarctic Thermolabile UDG 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.

Endonuclease Activity: Incubation of a 50 µl reaction in Standard *Taq* Reaction Buffer containing 15 units of Antarctic Thermolabile UDG with 1 µg φX174 RF I DNA for 4 hours at 37°C resulted in < 20% conversion to RFI as determined by agarose gel electrophoresis.

RNase Assay (Extended Digestion): A 10 µl reaction in NEBuffer 4 containing 40 ng of F-300 RNA Probe and a minimum of 1 unit of Antarctic Thermolabile UDG is incubated at 37°C. After

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incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Single Stranded DNase Activity (FAM Labeled Oligo): A 50 µl reaction in NEBuffer 4 containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 1 unit of Antarctic Thermolabile UDG incubated for 16 hours at 37°C yields < 5% degradation as determined by capillary electrophoresis.

Double Stranded DNase Activity (Labeled Oligo): A 50 µl reaction in NEBuffer 4 containing a 20 nM solution of a fluorescent labeled double stranded oligonucleotide containing a blunt end and a minimum of 1 unit of Antarctic Thermolabile UDG incubated for 16 hours at 37°C yields < 5% degradation as determined by capillary electrophoresis.

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DNase Activity (Labeled Oligo, 3' extension): A 50 µl reaction in NEBuffer 4 containing a 20 nM solution of a fluorescent labeled double stranded oligonucleotide containing a 3' extension and a minimum of 1 unit of Antarctic Thermolabile UDG incubated for 16 hours at 37°C yields < 5% degradation as determined by capillary electrophoresis.

DNase Activity (Labeled Oligo, 5' extension): A 50 µl reaction in NEBuffer 4 containing a 20 nM solution of a fluorescent labeled double stranded oligonucleotide containing a 5' extension and a minimum of 1 unit of Antarctic Thermolabile UDG incubated for 16 hours at 37°C yields < 5% degradation as determined by capillary electrophoresis.

Physical Purity: Purified to > 99% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

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qPCR DNA Contamination (*E. coli* Genomic): A minimum of 1 units of Antarctic Thermolabile UDG is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is < 1 copy of *E. coli* genome.

Heat Inactivation: 50°C for 5 minutes.

Reference:

1. Longo, M.C. et al. (1990) *Gene* 93, 125–128.



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