Uracil DNA Glycosylase (UDG)

RUO For Research Use Only

REF W6030

DESCRIPTION
Uracil-DNA Glycosylase (UDG) catalyzes the hydrolysis of the N-glycosylic bond between the uracil and sugar, leaving an abasic site in uracil-containing single or double-stranded DNA. The enzyme shows no measurable activity on short oligonucleotides (<6 bases), or RNA substrates.

KIT CONTENTS

Contents	W6030	W6030-5	W6030-10
Uracil DNA Glycosylase (5 U/µl)	200 µl	1,000 µl	2,000 µl
UDG Reaction Buffer (10X)	1 ml	5 ml	10 ml

APPLICATION
- Control of carry-over contamination in PCR
- Glycosylase mediated single nucleotide polymorphism detection
- Site-directed mutagenesis
- As a probe for protein-DNA interaction studies
- SNP genotyping
- Cloning of PCR products
- Generation of single strand overhangs of PCR products and cDNA

SOURCE OF PROTEIN
A recombinant *E. coli* strain carrying the Uracil DNA Glycosylase gene from E. coli K-12.

STORAGE BUFFER
10 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol pH 7.5 @ 25°C

10X UDG REACTION BUFFER
200mM Tris-HCl, 10 mM DTT, 10 mM EDTA, pH 8.0 @ 25°C

UNIT DEFINITION
1 unit is defined as the amount of enzyme that catalyzes the release of 1.8 nmol of Uracil in 30 minutes from double-stranded, tritiated, Uracil containing-DNA at 37°C in 1X UDG Reaction Buffer.

INHIBITION AND INACTIVATION
- Inhibitors: Ugi protein from the Bacillus subtilis phage PBS2, protein p56 from the Bacillus subtilis phage phi29.
- Inactivated by heating at 95°C for 10 min. Enzyme activity is partially restored at temperatures lower than 55°C. Therefore put PCR products on ice after PCR and load directly on a gel.

STORAGE CONDITIONS
- Store all components at -20°C in a non-frost-free freezer.

NOTE
- The abasic sites formed in DNA by Uracil-DNA Glycosylase may be subsequently cleaved by heat, alkali-treatment or endonucleases that cleave specifically at abasic sites.
- UDG is active in the presence or absence of divalent cations.

QUALITY CONTROL ANALYSIS:

Endodeoxyribonuclease Assay
No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 10 units of Uracil-DNA Glycosylase with 1 µg of pUC19 DNA for 4 hours at 37°C.

Ribonuclease Assay
No contaminating RNase activity was detected.

Labeled Oligonucleotide (LO) Assay
No degradation of single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 2 units of Uracil-DNA Glycosylase for 4 hours at 37°C.

SDS-Page (Physical Purity Assessment)
2.0 µl of concentrated enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µl of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

ORDERING INFORMATION

Product	Cat No.	Package
Uracil-DNA Glycosylase (UDG)	W6030	1,000 U
	W6030-5	5,000 U
	W6030-10	10,000 U

Technical Support

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