For general laboratory use.

Uracil-DNA Glycosylase, heat-labile

From marine bacterium BMTU 3346, recombinant in *E. coli*

Cat. No. 11 775 367 001 100 U
Cat. No. 11 775 375 001 500 U

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Store at −15 to −25°C

1. Product Description

Concentration

1 U/μl

Unit Definition

One unit is defined as the amount of Uracil-DNA Glycosylase required to completely degrade 1 μg purified single-stranded uracil-containing DNA (bacteriophage M13, grown in *E. coli* CJ236 dut^-ung^-) at +37°C in 60 min.

One Lindahl unit (1) is defined as the amount of enzyme necessary to release 1 μmol uracil at +37°C in 1 min. One Lindahl unit is comparable to 520,000 units based on our unit definition.

Enzyme Characteristics

Uracil-DNA Glycosylase (UNG) acts on single and double-stranded uracil-containing DNA (U-DNA) by hydrolysis of uracil-glycosidic bonds (base excision) at U-DNA sites, releasing uracil and creating an alkali-sensitive apyrimidinic (AP-DNA) site in the DNA (2). UNG is less active on double-stranded DNA than on single-stranded DNA. The enzyme is active on small U-DNA oligonucleotides and on dUMP, but has no activity on RNA or normal uracil-free DNA. Since Uracil-DNA Glycosylase has no metal ion requirements, the enzyme is active in the presence of Mg^{2+} or EDTA (2). However, glycerol, Mg^{2+}, and high ionic strength reduce enzyme activity.

The Uracil-DNA Glycosylase from BMTU 3346 is inactivated more quickly (2 min at +95°C) than the corresponding enzyme from *E. coli* (10 min at +95°C) (3). It has also been reported that UNG from *E. coli* remains partially active (or regains activity), leading to degradation of the dU-containing PCR product (9). In contrast to the enzyme from *E. coli*, the heat-labile UNG does not degrade dU-PCR products within at least several hours of incubation at +2 to +8°C (3). Therefore, it is not necessary to freeze the PCR product immediately after amplification or to hold the reaction mixture at +70°C.

Stability and Storage

If stored in storage buffer, Uracil-DNA Glycosylase, heat-labile, is stable at −15 to −25°C until the expiration date printed on the label.

Storage Buffer: 20 mM Tris-HCl, pH 8.0 (4°C), 0.1 mM EDTA, 100 mM KCl, 1 mM DTT, 50% glycerol (v/v), 0.5% Nonidet (v/v), 0.5% Tween 20 (v/v).

⚠ In buffers lacking stabilizers (*e.g.,* PCR buffer: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), the enzyme is rapidly inactivated at elevated temperatures (3).

Application

Uracil-DNA Glycosylase, heat-labile, can be used to cleave DNA at any site where a deoxyuridylate residue has been incorporated. The generated AP-DNA can then be hydrolyzed by alkali treatment, high temperature, or endonucleases that cleave specifically at apyrimidinic sites, such as T4 endonuclease.

Uracil-containing DNA (U-DNA) can be prepared by *in vitro* methods (2, 4). Site-specific, strand-specific, or general cleavage can be achieved with Uracil-DNA Glycosylase, depending on how the U-DNA is prepared.

Additionally, the enzyme can be used to increase the efficiency of site-directed mutagenesis procedures (5) and to produce highly labeled oligonucleotide probes (6).

Uracil-DNA Glycosylase can be used with dUTP to eliminate PCR carryover contamination from previous DNA synthesis reactions (7, 8). To make PCR products susceptible to degradation, dTTP must be substituted with dUTP in the PCR reaction mix. Subsequent PCR reaction mixes must be pretreated with Uracil-DNA Glycosylase (UNG) prior to PCR to degrade uracil-containing DNA. Native DNA does not contain uracil, therefore the sample is not degraded by this procedure.

⚠ Use LightCycler® Uracil-DNA Glycosylase (Cat. No. 03 539 806 001), in combination with LightCycler® reagents utilizing FastStart Taq DNA Polymerase.

2. Procedure

1	Replace dTTP with 200 μM – 600 μM dUTP in all your amplification reactions. ⚠ When using 600 μM dUTP, increase the $MgCl_2$ concentration to 2.5 mM.
2	Add 1 unit of Uracil-DNA Glycosylase to your reaction mix prior to starting your PCR.
3	Incubate the product for 10 min at +15 to +25°C.
4	Inactivate UNG by heating for 2 min at +95°C.
5	Perform PCR.
6	Store the PCR product at +2 to +8°C for up to several hours. For long-term storage, freeze the PCR product at −15 to −25°C.

3. Quality Control

Absence of Endonucleases

1 μg of uracil-free M13mp18ss-DNA (or uracil-free pBR322) is incubated in 50 μl SuRE/Cut Buffer L with Uracil-DNA Glycosylase for 16 hours at +37°C. Incubation with up to 20 U Uracil-DNA Glycosylase, heat-labile shows neither degradation of M13mp18ss-DNA nor relaxation of pBR322 DNA.

Absence of RNases

5 μg MS2 RNA are incubated with Uracil-DNA Glycosylase for 4 hours at +37°C in 50 μl SuRE/Cut Buffer L. Incubation with up to 10 U Uracil-DNA Glycosylase, heat-labile does not show RNase activity.

Absence of Exonucleases

Approximately 5 μg [^{3}H] labeled DNA are incubated with 3 μl Uracil-DNA Glycosylase, heat-labile for 4 hours at +37°C in a total volume of 100 μl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithioerythritol, pH approximately 7.5. Exonucleases are not detectable using up to 3 U Uracil-DNA Glycosylase, heat-labile.

PCR Carryover Prevention

Carryover prevention activity is assayed by adding approximately 10^5 molecules of dUTP containing template DNA prior to the amplification reaction. After UNG treatment, no amplification products can be detected.

4. References

1 Lindahl, T. *et al.* (1978) *J. Biol. Chem.* **252**, 3286 - 3294.
2 Duncan, B. K. (1981) DNA glycosylases, in Boyer (ed.): The enzymes, Academic Press, N.Y. pp. 565 - 586.
3 Sobek, H. *et al.* (1996) *FEBS Letters* **388**, 1-4.
4 Stuart, G. R. & Chambers, R. W. (1987) *Nucleic Acids Res.* **15**, 7451 - 7462.
5 Kunkel, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 488 - 492.
6 Craig, R.K. *et al.* (1989) *Nucleic Acids Res.* **17,** 4605 - 4610.
7 Kwok, S. & Higuchi, R. (1989) *Nature* **339,** 237.
8 Longo, M.C., Berninger, M.S., Hartely, J.L. (1990) *Gene* **93,** 125.
9 Thornton, Ch.G. *et al.* (1992) *BioTechniques* **13(2),** 180 - 183.
10 Jaeger, S. *et al.* (2000) *Extremophiles* **4(2),** 115-122.

5. Supplementary Information

5.1 Conventions

To make information consistent and memorable, the following text conventions are used in these Instructions for Use:

Text Convention	Use
Numbered Instructions labeled ➊, ➋, *etc.*	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols

In these Instructions for Use the following symbols are used to highlight important information:

Symbol	Description
ⓘ	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Ordering Information

Product	Pack Size	Cat. No.
dUTP, lithium salt	25 µmol (250 µl)	11 420 470 001
dUTP, PCR Grade	25 µmol (250 µl) 125 µmol (1,250 µl)	11 934 554 001 11 969 056 001
PCR Core Kit^{PLUS}	1 kit (50 PCR and UNG reactions)	11 585 541 001

5.3 Regulatory Disclaimer

For general laboratory use.

5.4 Disclaimer of License

For patent license limitations for individual products please refer to:
List of biochemical reagent products

5.5 Trademarks

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5.6 Changes to previous version

Editorial Changes.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany