Uracil-DNA Glycosylase

Cat. No. 11 444 646 001 100 U

Version 23
Content version: March 2020
Store at −15 to −25°C

1. Product Overview

Concentration

1 U/µl

Unit Definition

One unit is defined as the amount of Uracil-DNA Glycosylase necessary to completely degrade 1 µg purified single-stranded uracil containing DNA (bacteriophage M13, grown in *E. coli* CJ 236 dut$^-$ung$^-$) at +37°C in 60 min.

One Lindahl unit (2) is defined as the amount of enzyme necessary to release of 1 µmol uracil at +37°C in 1 minute. One Lindahl unit is comparable to 520,000 U based on our unit definition.

Storage and Stability

Stable at −15 to −25°C until the expiration date printed on the label.

⚠ Uracil-DNA Glycosylase remains partially active (<10%) after an incubation period of 30 min at +95°C. When using the enzyme for PCR carryover prevention, freeze the PCR product immediately after DNA synthesis. Under these conditions, U-DNA will not be degraded.

Storage Buffer

50 mM Hepes buffer, 0.3 M NaCl, 1 mM EDTA, 1 mM dithiothreitol, bovine serum albumin (BSA), 0.1 mg/ml, glycerol, 50% (v/v), pH 8.0.

Specificity

- Uracil-DNA Glycosylase hydrolyzes uracil-glycosidic bonds at U-DNA sites in single- and double-stranded DNA, excising uracil and creating alkali sensitive abasic sites in the DNA (1).
- The enzyme is more active on single-stranded DNA than on double-standed DNA.
- Activity was also observed on small U-DNA oligonucleotides and on dUMP (Duncan, unpublished observations).
- Uracil-DNA Glycosylase is inactive on RNA and native, uracil-free DNA.

Inhibitors

Glycerol, Mg^{2+} and high ionic strength buffers reduce enzyme activity.

⚠ Because Uracil-DNA Glycosylase has no metal ion requirements, it is fully active in the presence of EDTA (2).

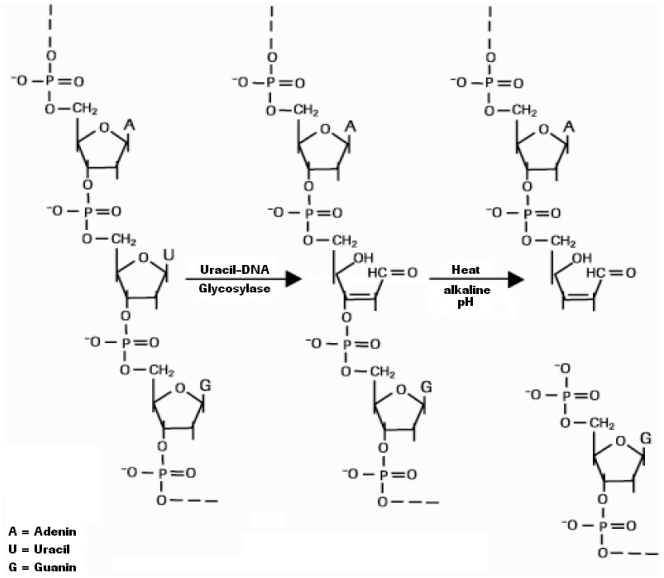

Fig. 1: Hydrolysis of uracil-glycosidic bonds at U-DNA sites and appropriate cleavage at alkaline pH and heat.

Application

- The enzyme can be used to increase the efficiency of site-directed mutagenesis procedures (4).
- The enzyme can be used in the production of highly labeled oligonucleotide probes (5).
- Uracil-DNA Glycosylase can be used with dUTP to eliminate PCR carryover contamination from previous DNA synthesis reactions (6, 7). 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 prior to PCR.

Description

Uracil-DNA Glycosylase can be used to cleave DNA at any site where a deoxyuridylate residue has been incorporated. The resulting abasic sites can then be hydrolyzed by:

- Alkali treatment
- High temperatures
- Endonucleases that cleave specifically at abasic sites.

U-DNA can be prepared by *in vitro* methods (1, 3).

General, site-specific, or strand-specific cleavage can be achieved with Uracil-DNA Glycosylase, depending on how the U-DNA is prepared.

2. How to Use This Product

2.1 Procedures and Required Material

Site-Directed Mutagenesis (SDM)

❶	Add 1 U Uracil-DNA Glycosylase to 50 µl reaction mix containing 1 µg uracil containing ss-DNA in 60 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, BSA, 0.1 mg/ml, pH 8.0.
❷	Incubate at +37°C for 60 min.
❸	Stop reaction by adding 16.5 µl 0.6 M NaOH.
❹	Incubate at +37°C for 5 min to hydrolyze AP sites.
❺	Neutralize by adding 16.5 µl 0.6 M HCl.
❻	Check degradation of ss-DNA by electrophoresis of an aliquot of the sample in a 1% agarose gel.

PCR Carryover Prevention

UTP-containing DNA is generated by PCR using a nucleotide concentration of 600 µM dUTP instead of 200 µM dTTP.

Increase the $MgCl_2$ concentration to 2.5 mM to gain optimal efficiency.

⚠ Using this application, uracil-containing DNA in the pg range (–10^7 molecules) is degraded.

❶	Add 1 U uracil-DNA glycosylase to a 100 µl PCR reaction mix.
❷	Incubate for 10 min at +15 to +25°C.
❸	Incubate for 10 min at +95°C to heat-inactivate Uracil-DNA Glycosylase.
❹	Start appropriate PCR cycling program. ⚠ Freeze sample immediately after the amplification step to avoid degradation of DNA by partially active Uracil-DNA Glycosylase.

3. Quality control

For lot-specific certificates of analysis, see section, **Contact and Support**.

References

1 Duncan, B. K. (1981) DNA glycosylases in Boyer (ed.) The enzymes, Academic Press, Y. pp. 565–586.

2 Lindahl, T. *et al.* (1977) *J. Biol. Chem.* **252**, 3286–3294.

3 Stuart, G. R. & Chambers, R. W. (1987) *Nucl. Acids Res.* **15**, 7451–7462.

4 Kunkel, T. (1985) Proc. *Natl. Acad. Sci. USA* **82**, 488–492.

5 Craig, R. K. *et al.* (1989) *Nucl. Acids Res.* **17**, 4605–4610.

6 Kwok, S. & Higuchi, R. (1989) *Nature* **339**, 237.

7 Longo, M. C., Berninger, M. S., & Hartley, J. L. (1990) *Gene* **93**, 125.

8 Jaeger, S. *et al.* (2000) *Extremophiles* **4**(2), 115-22.

4. Supplementary Information

Changes to Previous Version

- Update of the chapter Quality control.

Text Conventions

To make information consistent and understandable, the following text conventions are used in this Instruction Manual:

Text Convention	Use
Numbered instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed.

Symbols

Symbols are used in this Instruction Manual to highlight important information:

Symbol	Description
⚠	Important Note: Information critical to the success of the procedure or use of the product.

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
Uracil-DNA Glycosylase, heat-labile	100 U 500 U	11 775 367 001 11 775 375 001
PCR Core Kit[PLUS]	1 kit (50 PCR and UNG reactions)	11 585 541 001

Trademarks

EXPAND and FASTSTART are trademarks of Roche.

All third party product names and trademarks are the property of their respective owners..

Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

Disclaimer of License

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

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany