

# Uracil-DNA Glycosylase

Recombinant (*E. coli* K12)

Cat. No. 11 444 646 001

100 U

**Version 19.0**

May 2010

Store at -15 to -25° C

## Product overview

<b>Concentration</b>	1 U/μl
<b>Unit definition</b>	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 <i>E. coli</i> CJ 236 dut <sup>-</sup> ung <sup>-</sup> ) 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 520000 U based on our unit definition.

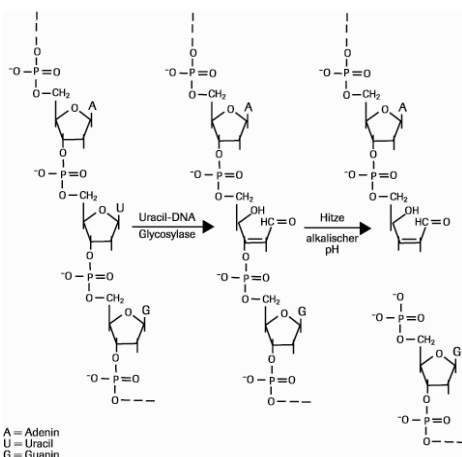
<b>Storage and stability</b>	Stable at -15 to -25°C until the expiration date printed on the label. <b>Note:</b> Uracil-DNA glycosylase remains partially active (<10%) after an incubation period of 30 min at 95°C. When using the enzyme for PCR carry-over prevention, it is therefore recommended to freeze the PCR product immediately after DNA synthesis. Under these conditions U-DNA will not be degraded.
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<b>Storage buffer</b>	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.
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<b>Specificity</b>	<ul style="list-style-type: none"> <li>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).</li> <li>The enzyme is more active on single-stranded DNA than on double-stranded DNA.</li> <li>Activity was also observed on small U-DNA oligonucleotides and on dUMP (Duncan, unpublished observations).</li> <li>Uracil-DNA glycosylase is inactive on RNA and native, uracil-free DNA.</li> </ul>
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<b>Inhibitors</b>	Glycerol, Mg <sup>2+</sup> and high ionic strength buffers reduce enzyme activity. <b>Note:</b> Because uracil-DNA glycosylase has no metal ion requirements, it is fully active in the presence of EDTA (2).
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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)
- Production of highly labeled oligonucleotide probes (5).
- Uracil-DNA glycosylase can be used with dUTP\* to eliminate PCR "carry-over" contaminations from previous DNA synthesis reactions (6, 7). To make PCR products susceptible to degradation, dTTP has to be substituted by 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 hydrolysed by

- alkali-treatment
- high temperatures or
- 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.

## Procedures and required material

### Site-directed mutagenesis (SDM)

Please refer to the following table.

Step	Action
1	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.
2	Incubate at 37°C for 60 min.
3	Stop reaction by adding 16.5 μl 0.6 M NaOH.
4	Incubate at 37°C for 5 min to hydrolyse AP sites.
5	Neutralize by adding 16.5 μl 0.6 M HCl.
6	Check degradation of ss-DNA by electrophoresis of an aliquot of the sample in a 1% agarose gel.

### PCR "carry over" prevention

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

We recommend to increase the MgCl<sub>2</sub> concentration to 2.5 mM to gain optimal efficiency.

**Note:** With this application, uracil containing DNA in the pg range (~ 10<sup>7</sup> molecules) is degraded.

Step	Action
1	Add 1 U uracil-DNA glycosylase to a 100 μl PCR reaction mix.
2	Incubate for 10 min at 15-25°C.
3	Incubate for 10 min at 95°C to heat-inactivate uracil-DNA glycosylase.
4	Start appropriate PCR cycling program. <b>Note:</b> Freeze sample immediately after the amplification step to avoid degradation of DNA by partially active uracil-DNA glycosylase.

## Quality control

**Incubation buffer** 60 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, bovine serum albumin (BSA), 0.1 mg/ml, pH 8.0.

**Absence of endonucleases** Treatment of 1 µg uracil-free M13mp18 ss-DNA or uracil-free pBR322 ds-DNA with more than 50 U of uracil-DNA glycosylase under standard assay conditions do not degrade the DNA.

## References

- Duncan, B. K. (1981) DNA glycosylases in Boyer (ed.) The enzymes, Academic Press, Y. pp. 565-586.
- Lindahl, T. et al. (1977) *J. Biol. Chem.* **252**, 3286-3294.
- Stuart, G. R. & Chambers, R. W. (1987) *Nucl. Acids Res.* **15**, 7451-7462.
- Kunkel, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 488-492.
- Craig, R. K. et al. (1989) *Nucl. Acids Res.* **17**, 4605-4610.
- Kwok, S. & Higuchi, R. (1989) *Nature* **339**, 237.
- Longo, M. C., Berninger, M. S. & Hartley, J. L. (1990) *Gene* **93**, 125.
- Jaeger, S. et al. (2000) *Extremophiles* **4**(2), 115-22.

## 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 (1250 µl)	11 934 554 001 11 969 056 001
Uracil DNA Glycosylase, heatlabile	100 U 500 U	11 775 367 001 11 775 375 001
PCR Core Kit <sup>PLUS</sup>	1 kit (50 PCR and UNG reactions)	11 585 541 001

## Note

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\*available from Roche Applied Science

## Regulatory Disclaimer

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

## PCR Product Selection Guide:

Needs	Size	Prevention of Carry over contamination*	Accuracy compared to Taq DNA Pol.	Use this RAS Science product	Pack size	Cat. No.
Standard PCR	up to 3 kb	Yes	1	Taq DNA Polymerase	100 U 500 U 4x 250 U 10x 250 U 20x 250 U	11 146 165 001 11 146 173 001 11 418 432 001 11 596 594 001 11 435 094 001
			1	PCR Core Kit <sup>PLUS</sup>	1 kit	11 585 541 001
		No	1	PCR Core Kit	1 kit	11 578 553 001
			1	PCR Master	1 kit	11 636 103 001
	up to 5 kb	Yes	6	Expand High Fidelity <sup>PLUS</sup> PCR System	125 U 2x 250 U 10x 250 U	03 300 242 001 03 300 226 001 03 300 234 001
			3	Expand High Fidelity PCR System	100 U 2x 250 U 10x 250 U	11 732 641 001 11 732 650 001 11 759 078 001
		No	3	High Fidelity PCR Master	1 kit	12 140 314 001
			1	FastStart Taq DNA Polymerase (Hot start)	100 U 500 U 4x 250 U 10x 250 U 20x 250 U	12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
Maximum specificity	up to 3 kb	Yes	1	FastStart Taq DNA Polymerase (Hot start)	100 U 500 U 4x 250 U 10x 250 U 20x 250 U	12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
	up to 5 kb	Yes	4	FastStart High Fidelity PCR System (Hot start)	125 U 2x 250 U 10x 250 U	03 553 426 001 03 553 400 001 03 553 361 001
High Fidelity PCR	up to 3 kb	No	18	Pwo SuperYield DNA Polymerase	100 U 2x 250 U	04 340 868 001 04 340 850 001
			18	Pwo Master	1 kit	03 789 403 001
			18	Pwo DNA Polymerases	100 U 2x 250 U	11 644 947 001 11 644 955 001
	up to 5 kb	No	3	Expand High Fidelity PCR System	100 U 2x 250 U 10x 250 U	11 732 641 001 11 732 650 001 11 759 078 001
			3	High Fidelity PCR Master	1 kit	12 140 314 001
		Yes	4	FastStart High Fidelity PCR System (Hot start)	125 U 2x 250 U 10x 250 U	03 553 426 001 03 553 400 001 03 553 361 001
			6	Expand High Fidelity <sup>PLUS</sup> PCR System	125 U 2x 250 U 10x 250 U	03 300 242 001 03 300 226 001 03 300 234 001
			3	Expand Long Template PCR System	150 U 2x 360 U 10x 360 U	11 681 834 001 11 681 842 001 11 759 060 001
Long Template PCR	5-20 kb	No	3	Expand Long Template PCR System	150 U 2x 360 U 10x 360 U	11 681 834 001 11 681 842 001 11 759 060 001
	> 20 kb	No	2	Expand 20 kb <sup>PLUS</sup> PCR System	200 U	11 811 002 001
Difficult templates & challenging assays	up to 3 kb	Yes	1	FastStart Taq DNA Polymerase (Hot start)	50 U	12 158 264 001
					100 U	12 032 902 001
					500 U	12 032 929 001
					4x 250 U	12 032 937 001
	up to 5 kb	Yes	4	FastStart High Fidelity PCR System (Hot start)	125 U	03 553 426 001
					2x 250 U	03 553 400 001
No	3	GC-RICH PCR System	100 U	12 140 306 001		
			150 U	11 681 834 001		
5-20 kb	No	3	Expand Long Template PCR System	2x 360 U 10x 360 U	11 681 842 001 11 759 060 001	

## PCR Nucleotide Selection

Product	Description	Pack size	Cat. No.
Set of Deoxy-Nucleotides, PCR Grade	Separate vials of dATP, dCTP, dGTP, and dTTP. 100 mM each	4x 25 µmol (4x 250 µl)	11 969 064 001
		4x 125 µmol (4x 1250 µl)	03 622 614 001
PCR Nucleotide Mix	Premixed ready-to-use solution of PCR Grade dATP, dCTP, dGTP, and dTTP. 10 mM each.	100 reactions 1000 reactions	11 581 295 001 11 814 362 001

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