For general laboratory use.

Uracil-DNA Glycosylase, heat-labile
from marine bacterium BMTU 3346

Version: 14
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Recombinant in *E. coli*.

Cat. No. 11 775 367 001	100 U 1 U/µl
Cat. No. 11 775 375 001	500 U 1 U/µl

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Uracil-DNA Glycosylase, heat-labile	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), 0.05% Tween 20 (v/v).	11 775 367 001	1 vial, 100 µl
			11 775 375 001	1 vial, 500 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Uracil-DNA Glycosylase, heat-labile	Store at −15 to −25°C. ⚠ ***In buffers lacking stabilizers, such as 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.***

1.3. Additional Equipment and Reagent required

For prevention of carryover contamination

- dUTP*

1.4. 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. 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 and to produce highly labeled oligonucleotide probes.

2. How to Use this Product

2.1. Before you Begin

General Considerations

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.
- 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, however, glycerol, Mg^{2+}, and high ionic strength reduce enzyme activity.

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. 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. Therefore, it is not necessary to freeze the PCR product immediately after amplification or to hold the reaction mixture at +70°C.

Prevention of Carryover Contamination

Uracil-DNA Glycosylase can be used with dUTP to eliminate PCR carryover contamination from previous DNA synthesis reactions.

1. To make PCR products susceptible to degradation, dTTP must be substituted by dUTP in the PCR reaction mix.

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2. 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* in combination with LightCycler® kits based on the FastStart Taq DNA Polymerase.***

2.2. Protocols

Prevention of PCR carryover contamination

1. Replace dTTP with 200 to 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 U Uracil-DNA Glycosylase to your reaction mix prior to starting your PCR.
3. Add the product for 10 minutes at +15 to +25°C.
4. Inactivate UNG by heating for 2 minutes 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.

2.3. Parameters

Inactivation

+95°C for 2 minutes.
Uracil-DNA Glycosylase from BMTU is inactivated more quickly than the corresponding enzyme from *E. coli* (10 minutes at +95°C).

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

3. Additional Information on this Product

3.1. Quality Control

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

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Removal of Nonidet P40 from product formulation and reduction of Tween 20 concentration from 0.5% to 0.05%. Information removed related to the REACH Annex XIV.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
LightCycler® Uracil-DNA Glycosylase	50 µL, 100 U, (2 U/µL)	03 539 806 001
dUTP	250 µl, 25 µmol, 100 mM 6,250 standard PCR assays of 20 µl each.	11 420 470 001
	250 µl, 25 µmol, 100 mM 6,250 standard PCR assays of 20 µl each.	11 934 554 001
	1,250 µl, 125 µmol, 100 mM 31,250 standard PCR assays of 20 µl each.	11 969 056 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM 125,000 using standard PCR assays of 20 µl each.	03 732 720 001

4.4. Trademarks

FASTSTART and LIGHTCYCLER are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.6. Regulatory Disclaimer

For general laboratory use.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or 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

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