For general laboratory use.

LightCycler® Uracil-DNA Glycosylase

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For use with FastStart enzyme-based LightCycler® Kits.

Cat. No. 03 539 806 001	50 µL 100 U, (2 U/µL)

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	LightCycler® Uracil-DNA Glycosylase	▪ Supplied in 50 µL Storage buffer at 2 U/µL. ▪ Storage buffer: contains 30 mM Tris-HCl, 0.15 M NaCl, 1 mM EDTA, 1 mM dithiothreitol, 0.05% Tween 20, glycerol 5% (v/v), pH 7.5 at −15 to −25°C.	1 vial, 100 U/50 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	LightCycler® Uracil-DNA Glycosylase	Store at +2 to +8°C for 4 weeks without a loss of activity. ⚠ ***Avoid repeated freezing and thawing (more than 5 times).*** ⓘ *To avoid temperature fluctuations, aliquot the enzyme and store at −15 to −25°C or store at +2 to +8°C for up to 4 weeks.*

1.3. Application

LightCycler® Uracil-DNA Glycosylase is used to prevent carryover contamination of PCR products that have been amplified in the presence of dUTP.

2. How to Use this Product

2.1. Before you Begin

General Considerations

LightCycler® Uracil-DNA Glycosylase can be used for decontamination together with LightCycler® Kits based on the FastStart enzyme. To make PCR products susceptible to degradation, dTTP is substituted by dUTP in all LightCycler® Kits. Subsequent PCR reaction mixes can be pretreated with LightCycler® Uracil-DNA Glycosylase prior to PCR.
LightCycler® Uracil-DNA Glycosylase is particularly suited for FastStart applications, since the 10 minute incubation at +95°C, which is necessary to heat-inactivate LightCycler® Uracil-DNA Glycosylase, is included in the FastStart cycling programs for activation of the FastStart enzyme.

Reaction conditions

LightCycler® Uracil-DNA Glycosylase is active from +15 to +55°C with an optimum at +50°C. The enzyme can be inactivated by heat treatment at +95°C for 10 minutes. This step is included within the protocols of the LightCycler® FastStart Kits.

- LightCycler® Uracil-DNA Glycosylase remains partially active, even after an incubation period of 30 minutes at +95°C.
- Since the enzyme is active up to +55°C, choose primers to allow the annealing temperature to be set at or above this level to prevent degradation of the newly synthesized dU-containing PCR products.
- The LightCycler® FastStart Kits can be combined with LightCycler® Uracil-DNA Glycosylase decontamination, even if lower annealing temperatures are used.
- When using the enzyme for PCR carryover prevention, store the PCR product at −15 to −25°C immediately after DNA synthesis, or add an equal volume of chloroform for inactivation of the LightCycler® Uracil-DNA Glycosylase, to prevent the U-DNA degradation.
- Short amplicons containing only few dU residues may not be degraded completely.

2.2. Protocols

Experimental protocol

DNA containing dUTP is generated by PCR using dUTP instead of dTTP. These conditions are given when using a LightCycler® FastStart Kit.
i With this application, uracil-containing DNA in the pg-range (-10^7 molecules) is degraded.

1. Prepare the LightCycler® Reaction Mix and Detection Mix as described in the corresponding kit protocols.

2. Add 0.5 U LightCycler® Uracil-DNA Glycosylase to the master mix per 20 µL final reaction.
   i The addition of more than 1 U LightCycler® Uracil-DNA Glycosylase to a 20 µL LightCycler® System reaction can lead to a shift in crossing-point values.

3. Add template, then incubate the completed reaction mixture for 10 minutes at +40°C.

4. Incubate for 10 minutes at +95°C to heat-inactivate the LightCycler® Uracil-DNA Glycosylase (this step is already included in the run program for the activation of the FastStart enzyme).

5. Start the appropriate PCR cycling program.
   i To avoid degradation of DNA by partially active or reactivated LightCycler® Uracil-DNA Glycosylase, store the sample at −15 to −25°C immediately after the amplification step.

2.3. Parameters

Inhibition

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

Because LightCycler® Uracil-DNA Glycosylase has no metal-ion requirements, it is fully active in the presence of EDTA.

Specificity

- LightCycler® Uracil-DNA Glycosylase hydrolyzes uracil-glycosidic bonds at U-DNA sites in single-stranded and double-stranded DNA, excising uracil and creating alkali- and heat-sensitive abasic sites in the DNA.
- The enzyme is more active on single-stranded DNA than on double-stranded DNA.
- LightCycler® Uracil-DNA Glycosylase is inactive on RNA and native, uracil-free DNA.

Unit Definition

One unit is defined as the amount of uracil-DNA glycosylase (UNG) that releases 1 nmol of uracil from a dUTP-containing DNA template into acid-soluble material per 60 minutes at +37°C.

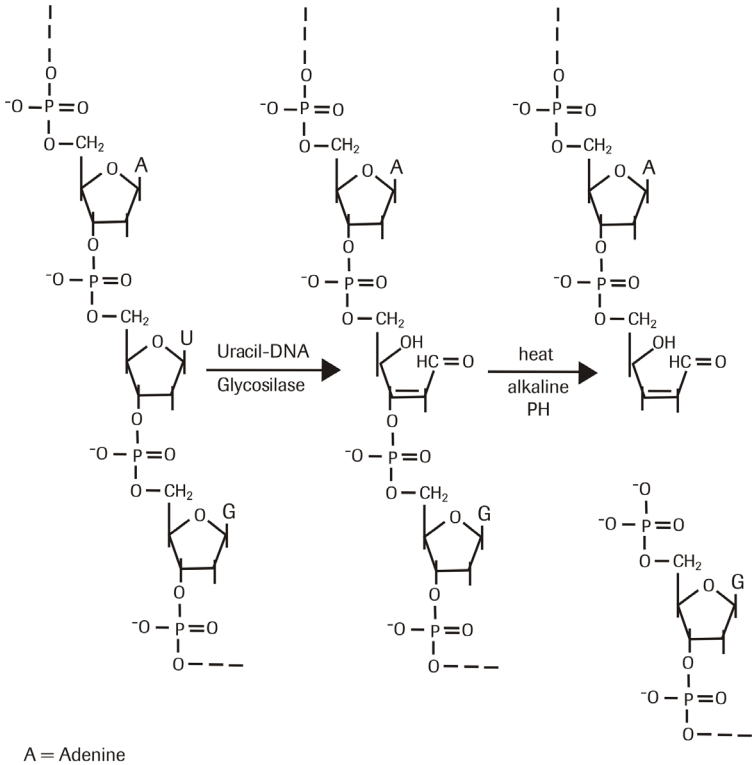
3. Additional Information on this Product

3.1. Test Principle

LightCycler® 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 (Duncan BK, 1981; Stuart GR, et al, 1987). General, site-specific, or strand-specific cleavage can be achieved with LightCycler® Uracil-DNA Glycosylase, depending on the U-DNA technique that is used.



A = Adenine
U = Uracil
G = Guanine

3.2. References

- Stuart GR, Chambers RW. Synthesis and properties of oligodeoxynucleotides with an AP site at a preselected position. Nucleic Acids Res. 1987;18:7451-7462.
- Duncan BK. 27 DNA Glycosylases. The Enzymes. 1981;14:565-586.

3.3. Quality Control

The LightCycler® Uracil-DNA Glycosylase is activity tested and function tested using the LightCycler® System.

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

Editorial changes.
Information about the LightCycler® PRO System has been added.
List of additional reagents and equipment has been updated.
Quality Control has been changed to LightCycler® System.

4.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a full overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Instruments		
LightCycler® PRO Instrument	1 instrument, 96-well version	09 541 713 001
	1 instrument, 384-well version	09 582 487 001
LightCycler® 96 Instrument	1 instrument	05 815 916 001
LightCycler® 480 Instrument II	1 instrument, 96-well version	05 015 278 001
	1 instrument, 384-well version	05 015 243 001
Reagents, kits		
LightCycler® EvoScript RNA Probes Master	1 kit, 200 reactions of 20 µl final volume each	07 800 029 001
	1 kit, 1,000 reactions of 20 µl final volume each	07 800 096 001
LightCycler® 480 Probes Master	5 x 1 ml, 2x conc. 5 x 100 reactions of 20 µl final volume each	04 707 494 001
	10 x 5 ml, 2x conc. 10 x 500 reactions of 20 µl final volume each	04 887 301 001
	1 x 50 ml, 2x conc. 5,000 reactions of 20 µl final volume each	04 902 343 001
FastStart Essential DNA Probes Master	1 kit, 500 reactions of 20 µL final volume each	06 402 682 001
	1 kit, 10 x 500 reactions of 20 µL final volume each	06 924 492 001
LightCycler® 480 SYBR Green I Master	5 × 1 ml, 2x conc. 5 x 100 reactions of 20 µl final volume each	04 707 516 001
	10 x 5 ml, 2x conc. 10 x 500 reactions of 20 µl final volume each	04 887 352 001
LightCycler® 480 High Resolution Melting Master	5 × 1 ml, 2x conc. 5 x 100 reactions of 20 µl final volume each	04 909 631 001
FastStart Essential DNA Green Master	1 kit, 500 reactions of 20 µL final volume each	06 402 712 001
	1 kit, 10 x 500 reactions of 20 µL final volume each	06 924 204 001
LightCycler® 480 Genotyping Master	4 × 384 µL, 5x conc. 384 reactions of 20 µL final volume each	04 707 524 001
LightCycler® FastStart DNA Master HybProbe	1 kit, 96 reactions of 20 µL final volume each	03 003 248 001
	1 kit, 480 reactions of 20 µL final volume each	12 239 272 001

4.4. Trademarks

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4.5. License Disclaimer

For additional documentation such as certificates and safety data sheets, please visit:
documentation.roche.com.

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

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