For general laboratory use.

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LightCycler® Uracil-DNA Glycosylase

Version: 06
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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 kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial/Bottle	Label	Catalog Number	Content
1	LightCycler® Uracil-DNA Glycosylase	03539806001	100 U, supplied in 50 µl storage buffer at 2 U/µl. **Storage Buffer** The 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).

1.2. Storage and Stability

Storage Conditions (Product)

LightCycler® Uracil-DNA Glycosylase is stable at −15 to −25°C until the expiration data printed on the label.

Vial/Bottle	Storage
1	Store at +2 to +8°C for 4 weeks without a loss of activity ⚠ ***Avoid temperature fluctuations (>5x)*** ⓘ *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 must 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 a maximum at +50°C. LightCycler® Uracil-DNA Glycosylase 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 LightCycler® Uracil-DNA Glycosylase is active at up to +55°C, primers should be chosen 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 available from Roche can be combined with LightCycler® Uracil-DNA Glycosylase decontamination, even if lower annealing temperatures are used.*
- *When using the enzyme for PCR carryover prevention, freeze the PCR product 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. Protocol

Experimental Protocol

DNA containing dUTP is generated by PCR using dUTP instead of dTTP. These conditions are given when using a LightCycler® FastStart Kit.

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.

   The addition of more than 1 U LightCycler® Uracil-DNA Glycosylase to a 20 µl LightCycler® 2.0 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.

   To avoid degradation of DNA by partially active or reactivated LightCycler® Uracil-DNA Glycosylase, freeze the sample immediately after the amplification step.

2.3. Other 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 (Lindahl et al.,1978).

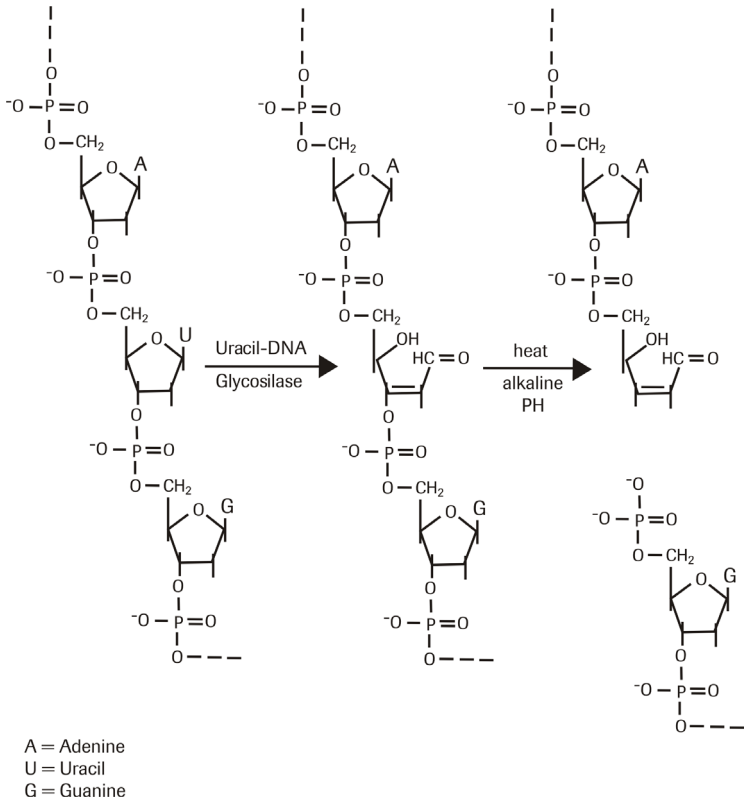
3. Additional Information on this Product

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 or
- endonucleases that cleave specifically at abasic sites.

U-DNA can be prepared by *in vitro* methods (Duncan, B. K.,1981; Stuart; G. R. 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.



3.2. References

- Duncan BK - DNA glycosylases (1981) , 565-586
- Lindahl T, Ljungquist S, Siegert W, Nyberg B, Sperens B - DNA N-glycosidases: properties of uracil-DNA glycosidase from Escherichia coli (1978) *Journal of biological chemistry* **10**, 3286-3294
- Stuart GR, Chambers RW - Synthesis and properties of oligodeoxynucleotides with an AP site at a preselected position (1987) *Nucleic Acids Res* **18**, 7451-7462

3.3. Quality Control

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

- No single-stranded or double-stranded endonuclease activities are detected when 5 units of LightCycler® UNG are incubated at +37°C for 1 hour with 1.2 µg of circular M13 DNA or 600 ng supercoiled pBR 322 (dcm^-, dam^-), respectively, in a final volume of 50 µl.
- No double-stranded exonuclease activity is detected when 5 units of LightCycler® Uracil-DNA Glycosylase is incubated at +37°C for 30 minutes with 1 pmol of [^{3}H]-dT-labeled 500-bp Lambda PCR product in a final volume of 50 µl.
- No 5'-single-stranded exonuclease activity is detected when 5 units of LightCycler® Uracil-DNA Glycosylase is incubated at +37°C for 30 minutes with 0.3 pmol of a 5' [α-^{32}P]-labeled oligonucleotide (41-mer) in a final volume of 50 µl.
- No 3'-single-stranded exonuclease activity is detected when 5 units of LightCycler® Uracil-DNA Glycosylase is incubated at +37°C for 30 minutes with an oligonucleotide (40-mer) labeled at the 3'-end with [α-^{32}P]-Cordycepin in a final volume of 50 µl.

3.4. Other Parameters

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 (Duncan, B. K.,1981).
- 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.

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

Layout Changes.
Editorial changes.

4.3. Ordering Information

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

Product	Pack Size	Cat. No.
Reagents , kits		
LightCycler® TaqMan® Master	1 kit, 96 reactions of 20 µl final volume each	04 535 286 001
	1 kit, 480 reactions of 20 µl final volume each	04 735 536 001
LightCycler® FastStart DNA Master^{PLUS} SYBR Green I	1 kit, 96 reactions of 20 µl final volume each	03 515 869 001
	1 kit, 480 reactions of 20 µl final volume each	03 515 885 001
	1 kit, 1,920 reactions of 20 µl or 384 reactions of 100 µl final volume each	03 752 186 001
LightCycler® FastStart DNA Master^{PLUS} HybProbe	1 kit, 96 reactions of 20 µl final volume each	03 515 575 001
	1 kit, 480 reactions of 20 µl final volume each	03 515 567 001
	1 kit, 1,920 reactions of 20 µl or 384 reactions of 100 µl final volume each	03 752 178 001
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
LightCycler® 480 SYBR Green I Master	5 x 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 Genotyping Master	4 x 384 µl, 5x conc., 384 reactions of 20 µl final volume each	04 707 524 001
LightCycler® 480 High Resolution Melting Master	5 x 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
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® 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

4.4. Trademarks

FASTSTART and LIGHTCYCLER are trademarks of Roche.
All third party 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: http://technical-support.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

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

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