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Uracil DNA Glycosylase

Cat. No. 18054-015 **Size: 100 units**

Conc. 1 U/µL **Store at -20°C (in a non-frost-free freezer)**

Description

Uracil DNA glycosylase (uracil-N-glycosylase) is the product of the *Escherichia coli ung* gene, and has been cloned (1), sequenced (2) and expressed in *E. coli* (unpublished observations). Uracil DNA glycosylase (UDG) removes uracil residues from DNA (single- and double-stranded) without destroying the DNA sugar-phosphodiester backbone, thus preventing its use as a hybridization target or as a template for DNA polymerases. The resulting abasic sites are susceptible to hydrolytic cleavage at elevated temperatures. Thus, removal of uracil bases is usually accompanied by fragmentation of the DNA.

Unit Definition

One unit catalyzes the release of 1 nmol of free uracil in one hour at 37°C from ^{3}H-poly-dU.

Contents

Storage Buffer	Unit Assay Conditions
30 mM Tris-HCl (pH 7.5)	20 mM Tris-HCl (pH 8.4)
150 mM NaCl	50 mM KCl
1 mM EDTA	5 mM $MgCl_2$
1 mM DTT	4 µg/mL ^{3}H poly(dU)$_n$; 7×10^{5} cpm/µg
0.05% (w/v) Tween 20®	Reaction volume: 50 µL
50% (v/v) glycerol	Incubation: 10 minutes at 37°C

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Part no. 18054015.pps MAN0001340 Rev. Date: 20 May 2010

Product Qualification

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available at www.invitrogen.com/support.

Use of UDG to Control PCR* Carryover Contamination:

To eliminate carryover contamination, perform following changes to standard PCR protocols:

- Substitute dUTP for dTTP in all PCR reactions.

  Note: The UDG protocol is not applicable unless the potential contaminants contain many uracil bases per molecule (4).

- Add 1 unit of UDG per 100 µL PCR reaction.

  Add UDG as a normal part of the reaction mixture prior to layering mineral oil. One unit of UDG will eliminate as much as 5 ng of carryover contaminant using the protocol outlined here.

- Incubate all PCR reactions at 37°C for 10 minutes prior to temperature cycling.

  The thermocycler can be conveniently programmed to include this additional step. UDG removes uracil bases from carryover contamination during this incubation.

- Increase the initial denaturation step of the first PCR cycle to 10 minutes at 94°C (high temperatures inactivate the UDG and break the contaminants into small fragments which have 5' and 3' phosphate termini).

- Add two additional cycles to the number usually employed.

  Note: Amplification may be somewhat less efficient when incorporating dUTP in place of dTTP (4). This phenomenon seems to be target-dependent and may lead to slightly decreased yields of product in some instances. Additional cycles allow normal yields of product to be obtained.

- Maintain a final temperature of 72°C at the completion of the temperature cycling protocol.

  Note: Under special conditions UDG has been observed to regain some catalytic activity following heat denaturation. While we have not seen this happen following PCR, it may be desirable to maintain the PCR products at a temperature at which the UDG protein remains inactive. Therefore, if a soak file is to be employed following standard amplification, set the soak file temperature at 72°C.

References

1. Duncan, B. K., and Chambers, J. A. (1984) *GENE 28*, 211.
2. Varshney, U., Hutcheon, T., and van de Sande, J. H. (1988) *J. Biol. Chem. 263*, 7776.
3. Lindahl, T., Ljungquist, S., Siegert, W., Nyberg, B., and Sperens, B. (1977) *J. Biol. Chem. 252*, 3286.
4. Longo, M., Berninger, M., Hartley, J. (1990) *GENE 93*, 125.

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