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Genaxxon BioScience
Uracil-DNA Glycosylase (UDG)

Cat #	Description
M3069.0200	200 units Uracyl-DNA Glycosylase (UDG).
M3069.1000	1000 units Uracyl-DNA Glycosylase (UDG).

Description:

E.coli uracil-DNA glycosylase (UDG) catalyzes the release of free uracil from uracil containing DNA. UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA, but not from oligomers (6 or less bases).

Specifications:

Concentration: 10 - 40 units/µL

Storage buffer: 20mM Tris/HCl, pH8.0, 50mM NaCl, 0.1mM EDTA, 50% glycerol.

Reaction buffer (10X): 200mM Tris/HCl, pH8.0, 10mM EDTA. This reaction buffer is NOT meant for PCR applications.

Unit definition: One unit is defined as the amount of enzyme required to release 60pmol of uracil per minute from double-stranded, uracil containing DNA.

Source: *E.coli*

Storage: -20°C

Usage:

In PCRs even minuscule amounts of a contaminant can be amplified and lead to a false positive result. Such contaminants are often course of previous PCRs (carry-over). Therefore researchers have developed methods to avoid such contamination.

One common strategy is substituting dTTP by dUTP during PCR, to produce uracil containing DNA (U-DNA). Treating PCR reaction mixtures with Uracil-DNA Glycosylase (UDG) prior to PCR and subsequent cleavage of apyriminic polynucleotides at elevated temperature (95°C) under alkaline conditions (during the initial denaturation step) will remove contaminating U-DNA from the sample. This method, of course, requires that all PCR-reactions in the lab have to be carried out with dUTP instead of dTTP.

Protocol

step	Action
1	Replace dTTP in all amplification reactions by 200-600µM dUTP. **IMPORTANT:** When using 500-600µM dUTP the MgCl2 concentration should be increased by 1mM. For example from 1.5mM to 2.5mM. Or from 2.5mM to 3.5mM.
2	Pipette 1 unit Uracil-DNA Glyosylase into the PCR-reaction mix before start of PCR.
3	Incubate 10 min. at 15°C to 25°C.
4	Inactivate UDG by incubation at 95°C for 10 min..
5	After PCR product may be stored some hours at 2°C to 8°C. For long term storage freeze at -20°C to prevent degradation due to UDG residual activity.

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NOTES: PCR products containing dU perform as well as those containing dT when used as hybridization targets or as templates for dideoxy sequencing.

PCR products containing dU can be cloned directly, if they are transformed into UNG-bacterial hosts.

A dU containing substrate is readily digested by some common restriction enzymes (e.g. Eco RI and Bam HI), while others show reduced activity (e.g. Hpa I, Hind II, Hind II).

We do not recommend the use of dU-containing DNA for protein binding or DNA-binding interaction studies.

References:

Longo, M.C., et al., Use of uracily DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene, 93, 125-128, 1990.

Related Products:

Genaxxon offers a complete range of nucleoside-5'-triphosphates in highly purified form in different convenient ready-to-use solutions. The dNTP-sets and mixes are ready-to-use for DNA-polymerisation reactions, all DNA labelling- and sequencing reactions.

Cat #	Description
M3015.4020	Set of 4x20µmol dA, dC, dG, dT solution, 100mM Na-salt in 200µL H2O.
M3015.4100	Set of 4x100µmol dA, dC, dG, dT solution, 100mM Na-salt in 1mL H2O.
M3015.4500	Set of 4x500µmol dA, dC, dG, dT solution, 100mM Na-salt in 5 x 1mL H2O.
M3016.4010	Mix of 4x10µmol dA, dC, dG, dT solution, 10mM Na-salt in 200µL H2O.
M3016.4050	Mix of 4x50µmol dA, dC, dG, dT solution, 10mM Na-salt in 1mL H2O.