# Uracil-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). Source**:** *Escherichia coli* (E.coli)

**Content**

| Ref No. | 111005 | 111025 | color |
|---|---|---|---|
| **Uracil-DNA-Glycosylase** | **200 units** | **1000 units** | **blue** |
| **Complete UDG* Reaction Buffer (10x)** | **1.0 mL** | **1.0 mL** | **red** |
| **Datasheet** | **1** | **1** | **--** |

***UDG Reaction buffer (10x) 200 mM TrisHCl pH8.0, 10 mM EDTA

**Application:** Uracil-DNA-Glycosylase is suitable for control and reduction of carry-over contamination in PCR.

**Concentration:**10 U/µL

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

**Quality Control**

- 98 % protein homogeneity in 10 % SDS-PAGE
- No detectable exo-/endonuclease activities

**Storage condition:** -20 °C

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**Protocol: Preventing Carry-over Contamination with Uracil-DNA Glycosylase**

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

One common strategy is substituting dUTP for dTTP during PCR amplification, to produce uracil-containing DNA (U-DNA).

Treating subsequent PCR reaction mixtures with Uracil-DNA Glycosylase (UDG) prior to PCR amplification 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

**Short protocol**

- Replace in all amplification-reactions dTTP by 200 - 600 µM dUTP. Important: When using 600 µM dUTP the MgCl2 concentration should be increased to 2.5 mM
- Pipette 1 U Uracil-DNA Glycosylase into PCR reaction mix before you start PCR.
- Incubate 10 min at 15 - 25°C
- Inactivate UDG by incubation at 95 °C for 10 min
- After PCR product may be stored some hours at 2 – 8 °C. For long term storage freeze at 15 – 25 °C to prevent degradation due to UDG residual activity

**Note the following when using dU-containing PCR products in downstream applications:**

- 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 III) on these substrates.

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