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roboklon

Uracil-N-Glycosylase

(non-thermolabile)

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(non-thermolabile)

Cat. No.	Size
E1250-01	200 units

Unit Definition:
One unit of the enzyme catalyzes the release of 1 nanomole uracil from an uracil-containing DNA template in 60 min at 37°C.

Inactivation Temperature (10 min):
95°C

Storage Conditions:
Store at -20°C

For selective digestion of uracil-labelled template DNA.

Description:

- Uracil-N-glycosylase (UNG) is a pure 26 kDa enzyme, derived by recombinant expression in *E. coli*.
- UNG is applied in PCR and real-time PCR assays for preventing carryover contamination from previously conducted PCR assays.
- The enzyme excises uracil residues from dU-containing DNA fragments, leaving abasic sites and rendering the DNA molecules susceptible to hydrolysis during the initial denaturation step.
- For labeling of PCR amplicons with uracil, dTTP must be partially or completely substituted by dUTP. Usage of a modified dNTP mix with dTTP being partially replaced by dUTP results in incorporation of uracil residues within PCR amplicons. Any accidential carryover of uracil-labeled PCR products to freshly assembled PCR assays (e.g. by aerosols or by contaminated pipette tips) introduces uracil-labelled amplicons, which can be selectively removed by initial UNG treatment.
- UNG treatment is performed within a single reaction step. An initial incubation at 50°C for 2 min at the onset of the cycling program will digest any accidentally introduced, uracil-labelled PCR product. Template DNA, which does not carry any uracil residues, remains intact and unaffected by UNG treatment.
- Usage of a "HotStart" DNA polymerase is strictly required, due to pronounced levels of polymerase activity at 50°C. Perpetual Taq DNA Polymerase (Cat. No. E2700) is a suitable "HotStart" enzyme preparation, which initially remains inactive at 50°C before being activated in the following initial denaturation step.
- Uracil-N-glycosylase is thermally inactivated by incubation at 95°C for 10 min.

Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 20°C), 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 50% [v/v] glycerol.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease as well as non-specific single- and double-stranded DNase activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis

UNG Digestion Protocol:

1. Add 0.25 units UNG for each 25 µl of the PCR reaction mix. 0.25 units UNG are required per 25 µl reaction volume, for a 50 µl reaction volume 0.5 units UNG are required.
2. For removal of uracil-labelled, contaminating PCR amplicons, include an UNG incubation step at 50°C for 2 min at the beginning of the PCR cycling program.
3. During the initial denaturation step UNG is inactivated. UNG requires at least 10 min incubation at 95°C to be inactivated. Upon completion of thermal inactivation, continue immediately with the PCR cycling program.
4. Upon completion of PCR amplification: Due to refolding, UNG activity may be partially restored at temperatures lower than 55°C. In case a dUTP-supplemented dNTP mix is used for setup of PCR reactions, it is recommended to perform all PCR steps, including annealing, at temperatures equal to or higher than 55°C. Upon completion of PCR, either cool reactions to 4°C and load directly on a gel, store assays frozen or purify DNA immediately.