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roboklon

Thermolabile Uracil-N-Glycosylase

Uracil-N-Glycosylase
(UNG, thermolabile)

Cat. No.	Size
E1251-01	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 (5 min):
50˚C

Storage Conditions:
Store at -20˚C

For selective digestion of uracil-labeled template DNA.

Description:

- Thermolabile Uracil-N-glycosylase (UNG) is a pure 27 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-labeled amplicons, which can be selectively removed by initial UNG treatment.
- UNG treatment is performed within a single reaction step. An initial incubation at room temperature to 37˚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" *Taq* DNA polymerase is strictly required, due to pronounced levels of polymerase activity at 37˚C. Perpetual *Taq* DNA Polymerase (Cat. No. E2700) is a suitable enzyme preparation. It is not possible to conduct UNG digestion on ice while using a non-"HotStart" *Taq* preparation.
- Thermolabile Uracil-N-glycosylase is thermally inactivated by incubation at 50˚C for 5 min. Thermal inactivation is irreversible, UNG activity is not restored due to partial refolding (as opposed to non-thermolabile UNG, Cat. No. E1250).

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 volume of PCR reaction mix. Examples: 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 room temperature or at 37˚C for 2 min at the beginning of the PCR cycling program. It is possible to include a dedicated incubation step for 2 min at 37˚C immediately before performing the initial denaturation step. But usually the time required for PCR reaction assembly suffices for completing the UNG digestion step.
3. UNG is inactivated during the initial denaturation step. UNG requires at least 10 min incubation at 50˚C to be inactivated.
4. Upon completion of thermal inactivation, continue immediately with the initial denaturation step of the PCR cycling program.