



Uracil-N-glycosylase

(UNG)

Cat. No.	size
E1250-01	500 u

Unit Definition:

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

Concentration:

1 U/μl

Storage Conditions:

Store at -20°C.

Inactivation Temperature:

10 min at 95°C.

Description:

- Uracil-N-glycosylase is a pure recombinant 26 kDa enzyme expressed in *E. coli*.
- UNG is used in PCR and real-time PCR to prevent carryover contamination between reactions.
- The enzyme removes uracil from any dU-containing contaminating amplicons, leaving abasic sites and making DNA molecules susceptible to hydrolysis during the initial denaturation step.
- To enable PCR amplicons to be degraded, dTTP must be partially or completely substituted by dUTP.
- The UNG treatment is performed at 50°C for 2 min at the onset of the cycling program.
- Uracil-N-glycosylase is 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% glycerol.

Procedure:

1. Add 0.25 units UNG for each 25 μl of the PCR reaction mix.
25 μl reaction requires 0.25 units UNG, 50 μl reaction requires 0.5 units UNG.
2. Include the UNG incubation step at 50°C for 2 min at the beginning of the cycling program.
3. During the initial denaturation step UNG is inactivated. UNG requires at least 10 min incubation at 95°C to be inactivated.
4. Follow the cycling program.
5. UNG activity may be partially restored at temperatures lower than 55°C due to refolding. It is recommended to perform PCR using a temperature equal 55°C or above for the annealing step. After completing the PCR cool reactions to 4°C and load directly on a gel or store frozen.

Quality Control:

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