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HK™-UNG Thermolabile Uracil N-Glycosylase

Cat. Nos. HU59100 and HU5901K

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1. Introduction

HK™-UNG Thermolabile Uracil N-Glycosylase, is a novel UNG enzyme that is easily **H**eat-**K**illed. Like standard UNG enzymes, HK-UNG hydrolyzes the N-glycosidic bond between the deoxyribose sugar and uracil in DNA that contains deoxyuridine in place of thymidine.[1] However, unlike standard UNG enzymes that are heat-stable (having significant activity remaining after treatment at 70°C-95°C), HK-UNG is thermolabile. While the enzyme is fully active at 37°C, 42°C, and 50°C, it is inactivated by a 10 minute incubation at 65°C or higher.

HK-UNG is active on both single- and double-stranded DNA that contains uracil, but has no activity on RNA or 2′-deoxyuridine-5′-monophosphate. The enzyme does not have AP endonuclease activity. Uracil-containing DNA can be synthesized *in vitro* with various DNA polymerases in reactions that contain dUTP in place of dTTP.[2]

HK-UNG is supplied with a dilution buffer for applications requiring lower concentrations of enzyme.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage & Dilution Buffers: HK-UNG is supplied in and with, a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1.0 mM dithiothreitol, 0.1 mM EDTA, and 0.1% Triton® X-100.

Unit Definition: One unit of HK-UNG catalyzes the release of 1 nmol of uracil from uracil-containing DNA in 1 hr at 37°C in 50 mM Tris-HCl (pH 9.0) and 20 mM ammonium sulfate.

Contaminating Activity Assays: HK-UNG is free of detectable exo- and endonuclease, and RNase activities.

3. Related Products

The following products are also available:

– dUTP Solution (20 mM)

4. Suggested Protocol for Degradation of DNA Containing Uracil

1. Briefly equilibrate samples containing DNA synthesized with dUTP at 37°C.
2. Dilute an appropriate amount of HK-UNG 10-fold with Dilution Buffer. Diluted enzyme may be stored for 2-4 weeks at –20°C in a freezer without a defrost cycle.
3. To a 50-µl reaction, add 1 µl (0.1 U) of the diluted HK-UNG.
4. Incubate at 37°C for 15-30 minutes to release uracil from the DNA.
5. Heat samples at 70°C for 10 minutes or 95°C for 3 minutes to break the sugar backbone and to completely inactivate the HK-UNG.

5. References

1. Lindahl, T. *et al.*, (1977) *J. Biol. Chem.* **252**, 3286.
2. Longo, M.C. *et al.*, (1990) *Gene* **93**, 125.

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