Uracil DNA Glycosidase (UNG Enzyme/UDG Enzyme)

Cat: U8181
Specification: 5U/μl, 100μl
Storage: Store at -20℃, and avoid repeated freezing and thawing.

Introduction

Bacterial UNG Uracil-DNA glycosylase, also known as Bacterial UNG, is derived from E. coli. UNG can catalyze the hydrolysis of the N-glycosidic bond between the uracil (dUTP) base and the deoxyribose in the DNA strand containing uracil, releasing free uracil. It can hydrolyze single-stranded or double-stranded DNA containing dUTP, but cannot hydrolyze RNA or DNA oligomers containing dUTP with a length of no more than 6 bases.

UNG is mainly used to avoid contamination problems during PCR amplification. The principle of preventing contamination is as follows: in the PCR reaction, an appropriate amount of dUTP and dTTP mixture is added to form a PCR product containing dUTP bases; When performing subsequent PCR reactions, use UNG enzyme to cleave single-stranded or double-stranded DNA containing dUTP that may have been contaminated and introduced during the previous PCR amplification process, to avoid the possible contamination of the previous PCR amplification products from affecting this PCR.

This product can be used in the conventional PCR or qPCR amplification detection system for DNA or cDNA, but it is generally not recommended for RT-PCR systems. Because under the reaction conditions of conventional reverse transcriptase, this UNG will maintain its hydrolytic activity and may digest newly synthesized cDNA.

Definition of enzyme activity: Definition of enzyme activity: the amount of enzyme that catalyzes the release of 1nM uracil from a DNA template containing uracil within 60 minutes at 37℃ is defined as one unit of activity U.

Storage Buffer：

20mM Tris-HCl，150mM NaCl，1mM EDTA，1mMDTT，0.05% Tween20，50% glycerol，pH8.0. 10× UNG Buffer：200 mM Tris-HCl (pH 8.2 at 25 °C), 10 mM EDTA, 100 mM NaCl.

Inactivation or inhibition:

Inhibitor: Ugi protein from the Bacillus subtilis phage PBS2 or p65 protein from the Bacillus subtilis phage phi29.

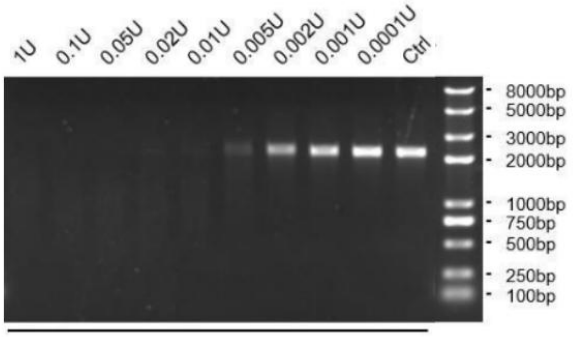
Deactivation: Heating at 95℃ for 10 minutes can deactivate more than 95% of UNG. When the temperature is below 55°C, the enzyme activity will partially recover. Therefore, after the PCR is completed, the PCR product should be placed on ice or directly subjected to gel identification.

Activity detection:

In a 10μl system, 100ng of PCR-amplified 2100bp DNA product containing dUTP bases was used as the substrate, and different amounts of 0U, 0.0001U, 0.001U, 0.002U, 0.005U, 0.01U,

0.02U, 0.05U, 0.1U and 1UUNG enzyme were added. In a 1× UNG Buffer system, the reaction was incubated at 37°C for 30min, and then subjected to 1% agarose gel electrophoresis for detection. As shown in the figure below, this product has considerable enzyme activity.Thermal stability test:

Incubate at 50 degrees for 10 minutes to inactivate. Then, in a 10μl system, 5μl of PCR amplification product containing 2100bp of dU base was used as the substrate, and different amounts of 0U, 0.0002U, 0.002U, 0.02U, 0.25U, 0.5U, and 1UHeat-labile Bacterial UNG were added. In a 1×Heat-labile Bacterial UNG Buffer system, the mixture was incubated at 37℃ for 30min, and then subjected to 1% agarose gel electrophoresis for detection. As shown in the figure, this product has considerable thermal stability.



UNG from Solarbio
Bacterial
1U/μl

Note：

1. This enzyme is active in most PCR or RT-PCR systems. It is recommended to test whether it is compatible with the system used before using it for the first time. Moreover, the activity of this product is not dependent on metal ions, but can be inhibited by high ionic strength (>200mM).
2. The enzyme may show residual activity at lower temperatures after heating denaturation due to refolding. Therefore, it is recommended to use a temperature of 55°C or higher during the annealing step for subsequent PCR.
3. If the enzyme needs to be applied to the RT-PCR system, reverse transcription and PCR need to be carried out in two steps. In reverse transcription, dUTP is not used; After reverse transcription, UNG enzyme treatment is used, followed by conventional PCR or qPCR.