Recombinant Uracil-DNA Glycosylase (Heat-labile UDG)

H292766

Storage at -20°C.Avoid freeze/thaw cycle.

Introduction:

The Recombinant Uracil-DNA Glycosylase (Heat-labile UDG) produced by our company, also known as Heat-labile Bacterial UDG (i.e., heat-labile bacterial UDG), is derived from the psychrophilic marine bacterium BMTU3346. It can catalyze the hydrolysis of the N-glycosidic bond between the uracil (dU) base and deoxyribose in the DNA strand containing uracil, thereby releasing free uracil. Uracil-DNA Glycosylase (UDG) can hydrolyze single-stranded or double-stranded DNA containing dU, but cannot hydrolyze RNA or DNA oligomers containing dU with a length of no more than 6 bases. UDG is mainly used to eliminate product contamination caused during PCR amplification. The principle of preventing contamination is as follows: an appropriate amount of dUTP is added to the PCR reaction, and dUTP is used to replace dTTP for incorporation into DNA to form PCR amplification products containing dU bases; during subsequent PCR reactions, UDG enzyme is used to selectively cleave the single-stranded or double-stranded DNA containing dU from previous PCR amplifications that may be introduced by contamination, thereby avoiding the negative impact of potential contamination from previous PCR amplification products on the current PCR amplification. This product is heat-labile and can be rapidly and irreversibly inactivated by incubation at 50℃ for 10 minutes. Before PCR or RT-PCR reactions, adding Heat-labile Bacterial UDG to the PCR or RT-PCR system and treating it at room temperature for 10 minutes can fully eliminate potential contamination from previous PCR amplification products containing dU. This product is not only suitable for PCR, but also for qPCR, RT-PCR, qRT-PCR and other systems.

Source	Recombinant expression in E. coli
Appearance	Sterile liquid
Storage Buffer	20mM Tris-HCl (pH 7.5), 50mM NaCl, 0.1mM EDTA, 1mM DTT, 50% (v/v) glycerol
Enzyme Concentration	1U/μL
Purity	Free of endonuclease, exonuclease, RNase, and phosphatase activities other than UDG enzyme activity
Activity Definition	One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 μl Standard Taq Reaction Buffer containing 0.2 μg DNA (10^4–10^5 cpm/μg) in 30 minutes at 37℃.

Components and Description:

H292766	Component	100U	5×100U	Storage
H292766A	Heat-labile Bacterial UDG (1U/μl)	100μl	5×100μl	-20℃. Avoid freeze/thaw cycle.
H292766B	10×Heat-labile Bacterial UDG Buffer	500μl	5×500μl	-20℃. Avoid freeze/thaw cycle.

Product Applications:

Removal of uracil bases from single-stranded or double-stranded DNA; removal of aerosol contamination from PCR products containing dU; in next-generation sequencing (NGS) applications, mainly used for the construction of mRNA-directed sequencing libraries; used to improve the efficiency of site-directed mutagenesis methods and obtain highly labeled oligonucleotide probes; used in PCR, qPCR, RT-PCR, RT-qPCR systems.

Product Advantages:

This product is heat-labile and can be rapidly and irreversibly inactivated by incubation at 50℃ for 10 minutes.

Instructions for Use:

Setup of PCR reaction system:

1. Set up the PCR or RT-PCR reaction system according to the conventional reaction system, and add Heat-labile Bacterial UDG to a final concentration of 0.02U/μl, then mix well. Usually, only the PCR or RT-PCR buffer needs to be added, and there is no need to add UDG buffer.
2. Incubate at 25℃ for 5-10 minutes (to remove potential contamination from PCR amplification products containing dUTP), then proceed to the PCR or RT-PCR program.

Matters needing attention:

1. Heat-labile Bacterial UDG enzyme is active in most PCR or RT-PCR systems. However, for self-used PCR or RT-PCR systems, it is recommended to test the compatibility with the used system for the first time. Usually, take the PCR amplification product containing dUTP, add an appropriate amount of UDG with reference to Figure 1, and observe whether the PCR amplification product containing dUTP can be effectively degraded. This product is inhibited by high ionic strength (>100mM).
2. For dNTP/dUTP, it is recommended to purchase D745378 dNTP/dUTP Mixture (2.5mM each/5mM) from Aladdin.
3. The activity of Heat-labile Bacterial UDG enzyme on double-stranded DNA is lower than that on single-stranded DNA. It has activity on small U-DNA oligonucleotides and dUMP, but no activity on RNA or normal DNA without dUTP.
4. The activity of Heat-labile Bacterial UDG enzyme is not dependent on metal ions, and it

has activity in the presence of Mg^{2+} or EDTA.

5. The abasic sites of the DNA strand generated by Heat-labile Bacterial UDG enzyme digestion can be removed by heating, alkali treatment, or endonuclease treatment. Usually, the heating step during the PCR reaction can ensure that the sites digested by UDG enzyme are completely cleaved.
6. Heat-labile Bacterial UDG enzyme can remove accidentally contaminated PCR products containing dUTP before the PCR reaction, thereby avoiding false positive PCR results caused by contamination.
7. If Heat-labile Bacterial UDG enzyme needs to be applied to One-Step RT-PCR or One-Step qRT-PCR systems, a thermostable reverse transcriptase should be selected, and the reverse transcription temperature needs to be set to 50-55℃.
8. This product is only for scientific research by professionals, and shall not be used for clinical diagnosis or treatment, nor for food or drugs. It shall not be stored in ordinary residences.
9. For your safety and health, please wear a lab coat and disposable gloves during operation.