Recombinant Uracil-DNA Glycosylase (UDG)

U292571

Storage: -20℃. Avoid freeze/thaw cycle.

Introduction:

The Uracil-DNA Glycosylase (E. coli) produced by our company, also known as E. coli Uracil-DNA Glycosylase (UDG) or E. coli Uracil N-Glycosylase (UNG) (i.e., E. coli UDG or UNG), 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.

Source	Recombinant expression in E. coli
Appearance	Sterile liquid
Storage Buffer	10mM Tris-HCl (pH 7.4), 50mM KCl, 0.1mM EDTA, 1mM DTT, 0.1mg/ml BSA, 50% (v/v) glycerol
Enzyme Concentration	5U/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 the release of [^{3}H]-uracil in a 50 μl reaction containing 0.2 μg DNA (10^4–10^5 cpm/μg) for 30 minutes at 37℃.

Components and Description:

U292571	Component	1KU	5×1KU	Storage
U292571A	E. coli UDG (5U/μl)	200μl	5×200μl	-20℃. Avoid freeze/thaw cycle.
U292571B	10× E. coli UDG Buffer	1ml	5×1mL	-20℃. Avoid freeze/thaw cycle.

Product Applications:

Prevention of cross-contamination of PCR products; single nucleotide polymorphism detection (GMPD); site-directed mutagenesis; research on protein-DNA interactions; SNP genotyping; cloning of PCR products; preparation of PCR products or cDNA with single-stranded overhanging ends.

Product Advantages:

Uracil-DNA Glycosylase (UDG) can hydrolyze single-stranded or double-stranded DNA containing dU and is also used to eliminate product contamination caused during PCR amplification.

Instructions for Use:

1. Setup of PCR reaction system:Set up the PCR reaction system with reference to the table below, or with reference to the PCR amplification system used, and add E. coli UDG enzyme to a final concentration of 0.01U/μl. Usually, only PCR buffer needs to be added, and there is no need to add UDG buffer.

Reagent	Volume	Volume	Final Concentration
Nuclease-Free Water	(18.325-x)μl	(36.65-y)μl	-
10× PCR Buffer (with Mg^{2+})	2.5μl	5μl	1×(1.5mM Mg^{2+})
dNTP/dUTP (2.5mM each/5mM)	2μl	4μl	0.2mM each/0.4mM
Primer mix (10μM each)	2μl	4μl	0.8μM
Template	xμl	yμl	10pg-1μg
Taq DNA Polymerase (5U/μl)	0.125μl	0.25μl	-
E. coli UDG (5U/μl)	0.05μl	0.1μl	-
Total volume	25μl	50μl	-

Note 1: According to experimental needs, the final concentration of dNTP/dUTP (D745378 dNTP/dUTP Mixture (2.5mM each/5mM) is available for purchase) can be adjusted between 0.2-0.6mM. The final concentration of magnesium ions can be adjusted between 1.0-4.0mM.

Note 2: For a 25μl PCR reaction system, the dosage of E. coli UDG (5U/μl) is generally 0.25-0.5U.

Note 3: For the dosages of template and primers, please refer to the instruction manual of A156186 Taq DNA Polymerase or the product instruction manual of the corresponding PCR system.

2. With reference to the above reaction system, add E. coli UDG, mix well, and incubate at 37℃ for 10 minutes (this step can effectively remove potential contamination from previous PCR amplification products containing dUTP). Then, you can immediately proceed to the PCR amplification program (ensure that the annealing temperature is not lower than 55℃). According to our actual test results, when the annealing temperature is not lower than 55℃,

the use of this product will not affect the yield of PCR amplification products.

Precautions:

E. coli UDG enzyme is active in most PCR reaction buffer 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, and observe whether the PCR amplification product containing dUTP can be effectively degraded.

(1) For dNTP/dUTP, it is recommended to purchase D745378 dNTP/dUTP Mixture (2.5mM each/5mM) from Aladdin.

(2) The abasic sites of the DNA strand generated by E. coli 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.

(3) E. coli UDG enzyme is active in a relatively wide pH range, with an optimal pH value of 8.0. The activity of E. coli UDG does not require divalent cations and is inhibited by high ionic strength (> 200 mM).

(4) E. coli UDG enzyme can remove accidentally contaminated PCR products containing dUTP before the PCR reaction, thereby avoiding false positive PCR results caused by contamination.

(5) E. coli UDG may exhibit residual activity at lower temperatures due to refolding after heat denaturation. Therefore, it is recommended to use a temperature of 55℃ or higher for subsequent PCR in the annealing step.

(6) E. coli UDG can be used in conventional PCR or qPCR amplification systems for DNA or cDNA, but it is generally not recommended for RT-PCR systems. Because under reverse transcription conditions, E. coli UDG usually remains active and may digest the newly synthesized cDNA.

(7) After treatment at 95℃ for 10 minutes, E. coli UDG enzyme still retains a small amount of activity. If it is desired to use it in an RT-PCR system, reverse transcription and PCR need to be performed separately: do not use dUTP during reverse transcription, add E. coli UDG enzyme for treatment after reverse transcription, and then perform conventional PCR or qPCR; alternatively, it is recommended to add a UDG inhibitor (such as Ugi protein from Bacillus subtilis phage PBS2 or p56 protein from phage phi29) to further inhibit the enzyme activity of E. coli UDG.

(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.