E.coli UDG(Uracil DNA Glycosylase)

Product description

E. coli uracil-DNA glycosylase (UDG) is expressed by recombinant E. coli strains cloned with the E. coli UDG gene and purified in multiple steps. E. coli UDG catalyzes the hydrolysis of N-glycosidic bonds between uracil bases and deoxyribose in single- or double-stranded DNA, releasing free uracil, but not from 6-base or shorter oligonucleotides. Inactive to RNA.

Components and storage conditions

Components	1000 U	5000 U
Uracil-DNA Glycosylase (UDG) (5 U/μL)	200 μL	1 mL
10 X UDG Reaction Buffer	1.5 mL	1.5 mL
Store the components at -20°C for 2 years.		

Experimental manipulation

E. coli UDG loses more than 95% of its activity when incubated at 95°C for 10 min. Since UDG remains partially active after heat treatment at 95 °C, it is recommended to add uracil glycosylase inhibitors (UGIs) to prevent product DNA degradation. E. coli UDG inhibitors: UGI protein from Bacillus subtilis phage PBS2 and p56 protein from Bacillus subtilis phage phi29.

1. Reaction system

Component	Volume (μL)	Final Concentration
10×PCR Buffer (Mg^{2+} Plus)	5	1×
Taq DNA Polymerase (5 U/μL)	0.5	0.05 U/μL
Forward Primer (10 μM)	2	0.4 μM
Reverse Primer (10 μM)	2	0.4 μM
dUTP (10 mM)	3	0.6 mM
dCTP / dGTP/ dATP/ dTTP (10 mM each)	1	0.2 mM each
Template DNA	Optional	-
Uracil-DNA Glycosylase (UDG) (5 U/μL)	0.2	1 U/50 μL
ddH_2O	Up to 50	

Note: a. The final concentration of dUTP can be adjusted between 0.2 - 0.6 mM and the final concentration of MgCl2 can be adjusted between 2 - 3 mM according to experimental needs; b. UDGase usage is generally 0.1 - 1 U in 50 μL reaction system.

2. PCR reaction procedure

Reaction temperature	Reaction time	The number of cycles	objective
37℃	10 min	1	Degradation contains U-templates
95℃	2 min	1	UDGase inactivation, template predenaturation
94℃	30 sec	30-35 Cycles	denaturation
50-72℃	30 sec		anneal
72℃	60 sec/kb		extend
72℃	7 min	1	Final extension

Notes

1. Enzyme Unit (U) Definition: The amount of enzyme required to catalyze the release of 60 pmol uracil from uracil-containing double-stranded DNA per minute is defined as 1 U. Activity was determined by detecting the amount of [^{3}H]-uracil released from a reaction containing 0.2 μg DNA (10^4 - 10^5 cpm/μg) in 50 μL over 30 minutes at 37°C.

2. Store reagents: 10 mM Tris-HCl、50 mM KCl、1 mM DTT、0.1 mM EDTA、0.1 mg/ml BSA、50% Glycerol（pH 7.4 @ 25°C）.

3. 1X UDG Reaction Buffer：20 mM Tris-HCl、 1 mM DTT、 1 mM EDTA (pH 8 @ 25°C)。

4. Scope of application: removal of single-stranded or double-stranded DNA uracil bases; Remove aerosol contamination of PCR products containing dU, thus avoiding PCR false-positive results due to contamination.

5. UDG is active over a wide pH range, with an optimal pH of 8.0, does not require divalent cations, and is inhibited by high ionic strength (> 200 mM).

6. This product is for scientific research purposes only.

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