Uracil-DNA Glycosylase (Low Temperature)

Please read the manual carefully before use.

Cat. No. LU201

Storage: at-20°C for two years

Enzyme Concentration: 1000 units/ml

Description

Low temperature Uracil-DNA Glycosylase (UDG) is a recombinant protein derived from psychrophile marine bacteria that have been modified to be expressed in *Escherichia coli*. UDG enzyme can effectively hydrolyze the N-glycosidic bond between the uracil (dU) base and deoxyribose in single or double-stranded DNA, thus releasing free uracil and producing pyrimidine deficiency sites, which are mainly used to eliminate the aerosol contamination caused by PCR products containing dU. This product is easily hydrolyzed and fractured at high temperatures or pH. Compared with conventional UDG enzymes, the product can operate at a lower temperature (20°C), i.e., when preparing qPCR or qRT-PCR reaction systems at room temperature, it can degrade contaminated dU-containing templates. This enzyme does not degrade dU-containing cDNA (DNA/RNA hybrid form) produced during reverse transcription. This product has high activity in most PCR reaction buffers and is suitable for PCR/qPCR, RT-PCR/qRT-PCR, and LAMP/RT-LAMP.

Applications

Remove uracil base from single-stranded or double-stranded DNA; prevent aerosol contamination of PCR products containing dU and improve the specificity of the reaction.

Kit Contents

Component	LU201-01	LU201-02
Uracil-DNA Glycosylase (Low Temperature)	100 units	500 units

Definition of Enzyme Activity

One unit (U) Uracil-DNA Glycosylase (Low Temperature) is defined as the amount of enzyme required to release of 60 pmol uracil from uracil-containing double-stranded DNA per minute.

Condition of detection: Activity is measured by release of [3H]-uracil in a 50 µl standard Taq reaction buffer containing 0.2 µg DNA (10^4-10^5 cpm/µg) in 30 minutes.

Enzyme Storage Buffer

20 mM Tris-HCl (pH 7.5@25°C), 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% (w/v) Triton X-100, 50% Glycerol.

Reaction System

This product UDG is compatible with most PCR reaction buffers, but its activity is inhibited at high ion concentrations (>100 mM). It is recommended to test the compatibility of the enzyme with the system used for the first time.

Procedures

1. Reaction Component (50 µl Standard Taq Reaction System)

Component	Volume	Final Concentration
DNA Template	Variable	Variable
F/R Primers (10 µM)	2 µl each	0.4 µM each
10×Taq Buffer (with $MgCl_2$)	5 µl	1×
dA/G/CTP (10 mM each)	1 µl each	0.2 mM each
dUTP	Variable	0.2~0.6 mM
UDG (Low Temperature) (1 U/µl)	Variable*	-
Taq DNA Polymerase (5 U/µl)	0.5 µl	2.5 U
Nuclease-free Water	To 50 µl	-
Total Volume	50 µl	-

*According to the needs of the experiment, the final concentration of dUTP can be adjusted from 0.2~0.6 mM, and the final concentration of $MgCl_2$ can be adjusted from 2~3 mM. The recommended usage of UDG enzyme is generally 0.1 U~1 U/50 µl reaction system.

2. Reaction Procedure*

Reaction Temperature	Time	Cycles	Target
25°C	2 min	/	Degradation of U-containing template
94°C	5 min	/	Deactivate UDG, denature template
94°C	30 sec	30~35 cycles	
50-60°C	30 sec		PCR amplification
72°C	30 sec/kb		
72°C	10 min		Extension thoroughly

*The PCR reaction procedure can be adjusted according to the needs of the experiment. For qPCR, the system is prepared at room temperature without 25°C for re-incubation, and normal reaction procedures can be followed.

Notes

• The UDG enzyme of this product is active in most PCR or RT-PCR systems, but it is recommended to test whether it is compatible with the system used for the first time. Usually, the amplified product containing dUTP is taken, the appropriate UDG enzyme is added (the enzyme quantity gradient could be set, such as 0U, 0.125U, 0.25U and 0.5U) , and observe whether the template could be effectively degraded after incubation at 25°C.

• The UDG enzyme of this product can remove inadvertently contaminated dU-containing products before PCR reaction, thus effectively avoiding false positives caused by dU aerosol contamination, but it has no activity in normal dUTP-free DNA or dU contained in DNA/RNA hybrid strands.

FAQs & Solutions

1. What types of nucleic acids are active for UDG enzyme?

UDG enzyme of this product can hydrolyze dU-containing double-stranded and single-stranded DNA, but cannot hydrolyze RNA, dU-free DNA, and DNA oligomers with dU lengths less than 6 bases.

2. Does the reaction temperature of UDG enzyme to degrade the dU - containing template must be 25°C?

The UDG enzyme of this product can function at room temperature, and the incubation time can be adjusted in the range of 25 ~ 50°C. If the room temperature preparation system takes a long time, it is possible to directly enter the PCR amplification procedure without additional incubation steps, because the contaminants have been degraded during the room temperature preparation system.

3. What is the recommended method and dosage of UDG enzyme?

This product has high activity in most PCR reaction buffers. UDG enzyme is added in the preparation system until the final concentration is 0.02 U/μl, that is, 1 U UDG enzyme is added in the 50 μl reaction system. But according to the experimental needs, the specific dosage can be adjusted.

4. Can UDG enzyme be used in RT-PCR/qRT-PCR or LAMP experiments?

Yes. Compared with conventional UDG enzymes, the UDG enzyme of this product has no activity against dUTP contained in DNA/RNA hybrid stranded, so it will not degrade newly synthesized dU-containing products.

Quality Control

Item	Standard
Appearance	Colorless and transparent
Molecular Weight	24.6 kDa
Purity	≥95%（SDS-PAGE）
Enzyme Concentration	1 U/μl
Exonuclease Activity	Not detectable
Endonuclease Activity	Not detectable
RNase Activity	Not detectable
Non-specific Nuclease Activity	Not detectable

For research use only, not for clinical diagnosis.

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